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STUDIES ON THE LIFE HISTORY AND HABITS OF THE BEET LEAFHOPPER [1]

[PRELIMINARY PAPER]

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INTRODUCTION

Much has been published concerning the distribution and history of the beet leafhopper and its relation to the curly-top disease of sugar beets, but no complete account of its life history and habits has appeared. The present paper gives a brief summary of observations bearing on these points made during the past few years at Jerome, Idaho, and in the sugar-beet growing regions of California.

DESCRIPTION

EGG

The egg when first laid is transparent, elongate, and slightly curved. The posterior end tapers gradually almost to a point. Length 0.0612 to 0.0696 mm.; average width 0.0182 mm.

As the embryo develops, faint spots which later become conspicuous eye spots appear on either side of the anterior end. During development the color of the egg changes from white to lemon yellow with a slight tinge of green.

NYMPH

The recently hatched nymph is nearly transparent, with a light yellow tinge in the thorax and abdomen. The antennæ are hairlike and more than half as long as the body. The head is wider than the thorax or abdomen and is the most distinctive characteristic of this instar.

After the first molt the nymph is more slender and the head and antennæ are not nearly so conspicuous. Average length 1.40 mm.; width 0.45 mm. Color usually milky white with a green tinge. Faint brown blotches may be distinguished on the thorax.

In the third instar there is more variation in the coloring. General color varying from yellow with light brown markings to almost black. The pattern made by the brown blotches does not seem to be constant, but the denser coloration on the thorax has been designated as a "saddle" (*3, p. 21*).[2] Length 1.99 mm.

[1] *Eutettix tenella* Baker, suborder Homoptera, family Jassidae.

[2] Reference is made by number (italic) to "Literature cited," p. 252.

The color variations in the fourth instar are similar to those of the third. A red coloration is often observed. The spines on the legs are more conspicuous than formerly, and the wing pads extend to the dorsal margin of the third abdominal segment. Length 2.30 mm.

After the fourth molt the nymph has a slender appearance and is nearly the size of the adult. The wing pads extend approximately to the dorsal margin of the fourth abdominal segment. Length 3.2 mm.

ADULT

In California, during the summer, adults of this species may be collected showing a gradation in color from light green with no markings to dark gray with numerous markings on the elytra (Pl. 42, A–C). In the fall the percentage of dark forms is much larger, and during the winter it is unusual to find a light form. Some of the winter forms appear almost black.

The following color details are given to show, to some extent, the extreme contrast in coloration:

LIGHT FORM (Pl. 42, A).—Front yellow, with faint, light brown, transverse stripes. Eyes gray, with occasional brown spots. Vertex green and lemon yellow, the yellow predominating. Pronotum green. Scutum deep yellow. Elytra hyaline with light brown venation. No pigment in the elytra. Tergum appearing as dark bands through the folded elytra.

DARK FORM (Pl. 42, B).—Front yellow, with irregular, testaceous, transverse bands. Eyes a mixture of red and brown, red usually predominating. Vertex fulvous, apical portion with a white band cut in center by a narrow dark band. Pronotum olive, except for ivory anterior band with several black spots. Scutellum with two square, black spots at basal angles. Elytra subhyaline, marked with black about as follows: Two large, almost circular spots on corium; apical portion and irregular black blotches on claval region. Nervures dark brown, with dark pigment on each side forming irregular bands.

RESEMBLANCE TO OTHER SPECIES

There should be little difficulty in distinguishing the beet leafhopper from other leafhoppers commonly found on sugar beets in California. Occasionally the darkest forms resemble some species of Agallia in coloration, but even a superficial examination will be sufficient to separate these two genera. These species of Agallia do not have the slender appearance of the beet leafhopper and are much slower in their movements. After a little experience in collecting it is possible to distinguish between the two genera by their movements. *Eutettix tenella* rarely, if ever, feigns death when disturbed; but some of the species of Agallia are almost certain to fall over on their backs and lie for some time as if dead. This habit is often an aid in collecting when the leafhoppers are not abundant and a careful search is necessary. One species, *Cicadula 6-notata* Fallen, may often be confused with the beet leafhopper, especially when individuals of the latter are mainly of the green coloration. The six spots on the vertex of *C. 6-notata* are usually plainly evident, however, and will serve to distinguish this species from *E. tenella*.

LIFE HISTORY AND HABITS

REPRODUCTION

During the summer season mating occurs within a few days after the last molt is accomplished, but during the fall this period is greatly prolonged. In Idaho adults were observed copulating in cages during the late fall as well as during the summer season. At Spreckels, Calif., mating continued throughout the winter. Unfertilized females have been known to lay sterile eggs under certain conditions, but parthenogenesis has never been observed.

The preoviposition period is comparatively long. In all experiments 15 to 17 days elapsed between the date the female reached maturity and the date the first eggs were laid. A much longer period is common, especially during the winter and early spring.

OVIPOSITION

Under normal conditions the eggs of the beet leafhopper are usually placed in the petiole or midrib of the sugar-beet leaf, beneath the fibrous strands and at a slight angle. They are invariably deposited one at a time, but often they are arranged in rows of from two to five, placed end to end so that they give the appearance of overlapping. It is almost impossible to find the recently deposited eggs in the petioles; but after the embryo has developed a little and the eye spots have appeared they are comparatively conspicuous. When deposited in the leaf tissue the eggs are more easily detected by the raised areas on the leaf surface. By transmitted light eggs in this position appear as small, transparent slits.

While apparently preferring the sugar beet as a plant in which to deposit its eggs, this leafhopper will oviposit in a large number of other plants. Fleshy or succulent species offer the most suitable conditions for oviposition. Russian thistle (*Salsola kali* var. *tenuifolia*), filaree (*Erodium cicutarium* and *E. moschatum*), *Chenopodium* spp. (especially *murale*), and *Atriplex* spp. are plants from which eggs have been most commonly noted hatching under natural conditions. Most perennial plants are too tough and woody to be suitable for this purpose, and it is doubtful if they are of any great importance as hosts during the egg-laying period.

Ball (*2*, *p.* 40) records the number of eggs deposited by a single female of this species as about 80. At Spreckels, Calif., the maximum number of eggs deposited by one female was 237, while at Riverside, Calif., the maximum was 247. Many difficulties were encountered in the conduct of these experiments, and it is probable that, given more favorable conditions, the females might have deposited a larger number of eggs.

Meteorological conditions influence greatly the incubation period. A maximum period of 52 days has been observed during the early spring and a minimum of 10 under most favorable conditions. During the

height of the egg-laying season the incubation period ranged from 10 to 15 days.

Seasonal variations in the development of the nymph are wide, due mainly to differences in temperature and food supply. The entire nymphal period ranged from 25 to 52 days, while from 4 to 10 days were required for the completion of each instar.

NUMBER OF GENERATIONS

Ball (*1, p. 93; 3*) states that the beet leafhopper is a single-brooded species and implies that such is the case for conditions even as far south as Glendale, Ariz. Experiments conducted at Spreckels, Calif., demonstrated that there were unquestionably at least two generations annually in that locality. Under conditions more favorable than was usual for this part of the Salinas Valley, a third and even a fourth brood were obtained. There was only one brood on sugar beets in southern Idaho, but it seems probable that further investigation would reveal an additional brood, possibly on the wild vegetation.

LONGEVITY OF ADULTS

Under natural conditions it is doubtful if the normal length of life of the adult is more than 4 or 5 months. Fall-brood adults are not found in the fields during the summer, and the spring brood is rarely noted in the fall. Females have been kept alive in cages for 19 months, but it is doubtful if they would ever survive so long under field conditions.

SEASONAL HISTORY

IN SOUTHERN IDAHO

Although persistent effort was made to locate adults of the beet leafhopper during the winter and early spring in southern Idaho, they were not observed until their appearance on the sugar beets. The earliest record for this was June 6, 1914, when several individuals were collected on volunteer sugar-beet plants at Jerome. Apparently the leafhoppers are in the cultivated fields as soon as the beets are up.

Oviposition begins in the field as soon as the adults appear. Records have been made as early as June 22, when the beets were still young and had not yet been thinned. June 28 was the earliest hatching record obtained in cage experiments. Starting thus, early in June, oviposition continues throughout the season until late in October.

During 1913 adults were not observed copulating until late in the fall. On October 12, a large number of adults confined in a lantern globe were noted copulating for several days. During the one winter spent by the writer in this district only a few adults placed in cages in the fall survived the winter, and all of these were females. These observations indicate that the females are fertilized in the fall before hibernation and that a large percentage of males perished during the winter.

Weather conditions were severe enough during the winter in this district to necessitate hibernation. All attempts to determine the method of hibernation, however, as well as the places in which it takes place were failures. Adults in cages survived the winter underneath dead beet leaves and in the crown of the plant.

IN CALIFORNIA

Under California conditions adults and nymphs are most abundant in the field during August. At harvest time they are scattered, and no doubt a large number perish. After the beets have all been removed from the fields the leafhoppers seem to be greatly diminished in numbers, although they may be collected from certain weeds growing in the fields and along the irrigating canals. No indications of a general migration have been noted at such times, so it is assumed that the surviving individuals scatter over wild vegetation, selecting that which is most suitable for food and protection. Later they may congregate in certain spots which furnish especially favorable conditions during winter.

There is no true hibernation in the districts of California that have been under observation. Adults have been collected every week in the winter under conditions indicating that they were feeding when captured. Under cage conditions food must be available at all times. As a rule, all individuals kept without food died within 48 hours.

The characteristic dark-colored individuals of the fall brood that leave the beet fields could hardly be confused with the light-colored adults that appear the next spring. A small percentage of the fall-brood adults may remain in or near the beet fields during the winter and be responsible for the early injury in the spring, but it is usually not until the light forms appear in considerable numbers that attention is directed to the damage. The striking difference in coloration between the fall and spring forms suggests at once the possibility of a new brood on wild vegetation before migration into the beet fields. Observations and cage experiments have proved that such a brood occurs.

The time when the leafhoppers first appear in fields in spring in California varies with the seasonal conditions in different localities, being from April 1 to June 1. The condition of wild vegetation in the natural breeding areas is an important factor in determining when migration to the beet field will take place. As long as this vegetation is abundant and succulent it is doubtful if there is any general movement into the cultivated areas.

Oviposition begins as soon as the adults appear in the field and continues throughout the season. There is an overlapping of broods which makes it impossible to determine the exact number under field conditions. Cage experiments, however, have demonstrated that there may be from one to three each year on the beets. Thus the maximum number of broods in one year would be four.

NATURAL ENEMIES

EGG PARASITES

The following three species of egg parasites have been reared from the beet leafhopper and studied to some extent. They are given in the order of their importance.

POLYNEMA EUTETTIXI GIRAULT (*4, p. 18*) (PL. 43, A).—This small brown or black species was first reared from eggs of *Eutettix tenella* at Spreckels, Calif., early in 1915 and has proved to be the most effective parasite of this group in the Salinas Valley. Eggs parasitized by this species are conspicuous in the petioles of the beets because of the black color of the parasite pupæ. Development is rapid, the life cycle from adult to adult covering about 35 days on an average, and there are at least nine generations annually.

ABBELLA SUBFLAVA GIRAULT.—Concerning this parasite W. J. Hartung (*5*) writes as follows:

> Hyper-parasites were bred from parasitized eggs of Eutettix. These were determined by Girault as *Abbella subflava* Girault.

This species[1] was never found among the parasites reared from material collected at Spreckels, Calif., but at Riverside it was reared in about equal numbers with *Polynema eutettixi*.[1] It is a primary parasite, ovipositing readily in eggs of the beet leafhopper. It has also been reared from eggs of *Empoasca* sp.

ANAGRUS GIRAULTI CRAWFORD.—This common orange or red jassid egg parasite has been reared in each locality where parasite studies have been conducted. It oviposits readily in eggs of the beet leafhopper and is usually reared along with *Polynema eutettixi*, but not in such large numbers. The presence of this species in the petioles of the beet can be detected by the red or orange color found in both larva and pupa.

PARASITES OF THE NYMPHS AND ADULTS

As previously reported by Hartung and Severin (*6*), two species of the dipterous family Pipunculidae are known to be parasitic on the nymphs and adults of the beet leafhopper. These have been described (*7*) as *Pipunculus industrius* Knab and *Pipunculus vagabundus* Knab. The former is the more common species in the Salinas Valley.

PIPUNCULUS INDUSTRIUS KNAB (PL. 43, B).—Eggs of this species are deposited in both nymphs and adults of the beet leafhopper, but mature larvæ have never been known to emerge from a nymph. There are no indications that the adult female prefers either the mature or immature stages of the host in which to deposit her eggs, very small parasitic larvæ having been dissected in about equal numbers from both stages. It is known, by dissection, that eggs may be deposited in small nymphs

[1] Specimens identified by Mr. A. B. Gahan.

no further developed than the third instar. In all instances, however, where an action thought to be oviposition was observed, the adult host was the victim.

The adult is very graceful in flight, darting here and there so suddenly that it is impossible to follow the movements with the eye. The beet leafhopper, also, is very quick in its movements, but none is quick enough to avoid this active little parasite.

PIPUNCULUS VAGABUNDUS KNAB.—This species is not common in the Salinas Valley and is of little importance. Its habits are similar to those of *Pipunculus industrius*, and, with the exception of the conspicuous stigma which is absent in the wings of *P. vagabundus*, the two species are similar in appearance.

DRYINIDAE.—Occasionally beet leafhoppers, both adults and nymphs, are found with a dark brown sac or pouch protruding from the abdomen (Pl. 42, D). This pouch contains the larva of a dryinid parasite. Hartung and Severin (6) report a parasite of this family, *Gonatopus contortulus* Patton, from the Salinas Valley. Although the writer has reared many specimens of this family, none has been determined. Judging from the number of parasitized leafhoppers collected, these dryinids are not of much economic importance. It has been observed, however, that the adults devour a larger number of the leafhoppers, especially nymphs, than they parasitize. In this way they may be of more importance than would at first appear.

SUMMARY

Eggs of *Eutettix tenella* are deposited in a wide range of cultivated and wild plants, but the sugar beet seems to be preferred for this purpose during the summer season. A maximum record of 247 eggs was obtained for a single female. The incubation period covered from 10 to 15 days during the height of the egg-laying season and the nymphal period from 25 to 52 days.

One generation only was observed in southern Idaho, while from two to four were observed under California conditions.

In southern Idaho the beet leafhopper appears in the beet fields in June and starts reproducing at once, oviposition continuing throughout the season. After harvest the leafhoppers enter a true hibernation period.

In California the adults appear in the beet fields soon after April 1 and remain until harvest time, when they disperse to wild vegetation suitable for food and protection. No true hibernation was noted in California.

Three species of egg parasites were reared and studied. Two of these are very effective. Two species of Pipunculus, internal parasites of the nymphs and adults, were reared; and one of these was quite effective. Dryinid parasites, also, were reared but are not considered very efficient.

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PLATE 42

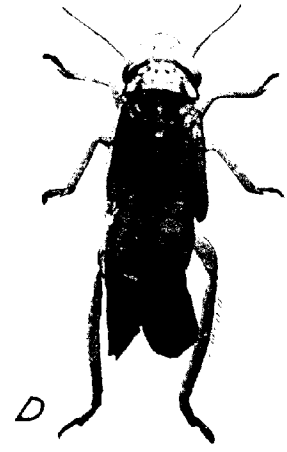
Eutettix tenella:

A.—Adult, light form.
B.—Adult, dark form.
C.—Adult, color gradation between A and B.
D.—Nymph with protruding sac of dryinid parasite.
All much enlarged.


A


B


C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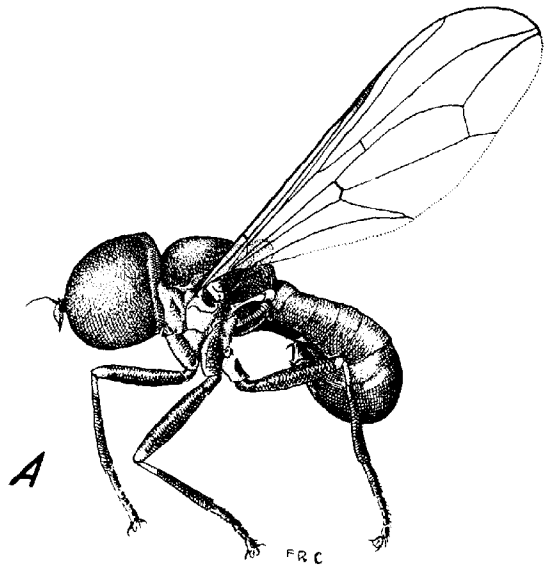
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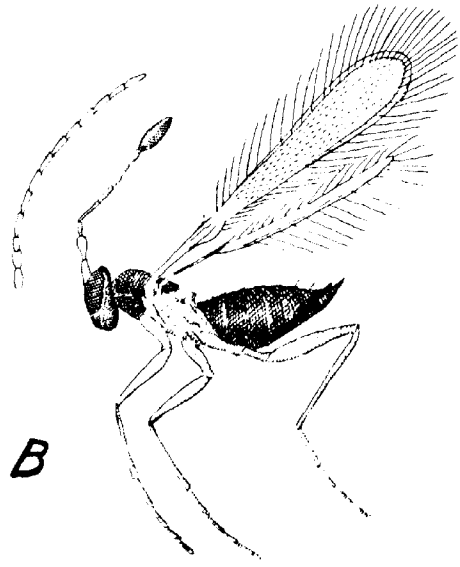
B

PLATE 43

Parasites of *Eutettix tenella:*

A.—*Pipunculus industrius:* Adult, much enlarged.
B.—*Polynema eutettixi:* Adult, much enlarged.

HYPERTROPHIED LENTICELS ON THE ROOTS OF CONIFERS AND THEIR RELATION TO MOISTURE AND AERATION

By GLENN G. HAHN, *Scientific Assistant*, CARL HARTLEY, *Pathologist*, and ARTHUR S. RHOADS,[1] *Assistant in Forest Pathology, Investigations in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

At the Bessey Nursery of the United States Forest Service at Halsey, Nebr., warty excrescences were observed upon the roots of coniferous seedling stock during the shipping season of 1915. Such excrescences occurred on all pine species grown there. They were so abundant on western yellow pine (*Pinus ponderosa*)[2] that the possibility of a parasite as the causal agent was suggested, and the forest officers properly questioned the advisability of shipping the stock to other regions.

Attempts were made by the writers to obtain evidence of a pathogenic organism, but always with negative results. This experimentation consisted of (a) incubation in moist chambers of portions of roots bearing excrescences, (b) insertion of the interior portion of the excrescences, removed with aseptic precautions, into nutrient agar, and (c) inoculation of portions of the excrescences into roots of healthy 2-year-old and 4-year-old *Pinus ponderosa* stock.

After the failure to obtain evidence of a pathologic organism, a histological examination was made, which showed that the excrescences had the structure of the hypertrophied lenticels (Pl. 44) so commonly seen in many dicotyledonous plants.

DESCRIPTION

The hypertrophied lenticles are found both upon the main tap root (Pl. 45, B) and upon the lateral roots, not only close to the ground level and upon the stems proper but also on the tap roots as far as 14 inches (36 cm.) below the surface of the soil.[3] On the stems of conifers the hypertrophied lenticles usually occur only on the basal portions of trees growing in abnormally wet situations (Pl. 45, A) or on parts otherwise submerged. In exceptionally humid situations they may occur occasionally on parts of the stems above the soil surface.

[1] The writers wish to acknowledge helpful suggestions from Dr. B. E. Livingston, of the Johns Hopkins University, and Dr. T. H. Goodspeed, of the University of California.

[2] All the western yellow pine referred to in this paper was the type sometimes referred to as *Pinus ponderosa* var. *scopulorum*, from eastern Rocky Mountain seed.

[3] In all probability hypertrophied lenticels will be found at much greater soil depths on the roots of older trees.

On the small roots the hypertrophied lenticels occur most commonly, but not always, at the junction of a lateral root or rootlet with its parent root, usually originating immediately above the point of origin but also subtending, at the sides or immediately below, the root or rootlet in question. This agrees with the findings of De Vaux (5)[1] on normal lenticels, who reports that primary lenticels on roots are always at the bases of root branches, though secondary lenticels are sometimes formed later at other points. It was this coincidence of lenticels and root branches that caused some botanists during the early part of the nineteenth century to believe lenticels equivalent to buds, a doctrine attributed to De Candolle (*7; 13, Vorwort*) and overthrown by Majer (*13*),[2] Unger (*22*), Terras (*19*), and others.

The excrescences vary greatly in size and shape, from minute circular areas 0.5 mm. in diameter to bands nearly encircling the larger roots in cases where two or more lenticels have become laterally confluent. Around the root crowns and the bases of the submerged stems large, wartlike patches may occur, 5 to 8 mm. in diameter and projecting 1 to 3 mm. above the surface of the bark. Examination with a dissecting microscope shows these excrescences to be made up of a very loosely piled mound of pale yellowish tissue. As a general rule these mounds of loosely piled cells split in a stellate manner, the segments recurving outward, occasionally leaving a few filamentous columns standing by themselves in the center. Such structure is evident only when the young trees have been removed from the ground with great care, for the slightest touch upon these loose-lying columns causes them to crumble instantly to a flat, powdery mass, especially when they are dry. On the bases of still older stems 1 to 2 inches (2.5 to 5 cm.) in diameter that stand for a large part of the growing season in water or poorly drained soil, the bark, which is here considerably thickened, exfoliates in patches of varying size, revealing irregularly connected flattened masses of cells, or, more rarely, unbroken areas of such cells 1 inch (2.5 cm.) broad. On some pines these excrescences frequently become so abundant that considerable areas of the lower stem and the tap root are covered by them (Pl. 46, B). After the cessation of growth in the lenticels, these excrescences become dark root-brown and gradually slough off.

The lenticellular excrescences vary in different conifers from loosely connected, more or less divergent, columnar masses crumbling at the slightest touch, common in the pines, to fairly compact, corky masses usually seen in the trees of other coniferous genera.

Histological examination of the excrescences at once proves the white, spongy tissue to consist of more or less loosely connected masses of cells developed from the phellogen. Plate 44 illustrates a cross section of

[1] Reference is made by number (italic) to "Literature cited," p. 264–265.

[2] This seems to be the 1836 paper attributed to Mohl by Haberland (*7*). Mohl apparently directed the work of Majer and wrote a preface for the dissertation, but Majer was the author of the paper itself.

one of these hypertrophied lenticels on a root of *Pinus rigida*. The outgrowths consist of homogeneous parenchymatous elements, more or less radially elongated, sometimes very much so. The individual cells are thin-walled with a thin layer of cytoplasm.

SPECIES AFFECTED

Stahl (*18*) states that all trees which have lenticels on the stems also have them on the roots. De Vaux (*5*) reports the presence of lenticels on the roots of a large number of tree species, including a number of conifers. For one species of Ephedra he states that lenticels are found only on the roots. He states that especially in *Pinus maritima* the lenticels on the roots are larger than those on the stems. This author was able to find or to produce lenticel hypertrophy on some part of the plant in 60 per cent of the 155 plant species considered but was unable to secure any hypertrophy on the representatives of the several coniferous genera which he studied. On roots less than 3 mm. in diameter he found the normal lenticels so small that the microscope was usually necessary in demonstrating them. Tubeuf (*20*) lists a small number of species, of which he was able to secure lenticel hypertrophy on some part of 12 nonconifers. He, however, failed to get this hypertrophy on species of Sequoia, Thuja, and Taxus, or on *Gingko biloba* and 14 other nonconiferous species. Zach (*23*) later secured hypertrophy of lenticels on stems of *G. biloba* under certain conditions. However, a rather careful search in the earlier literature appears to justify the statement by the reviewer of Zack's paper (*16*) that no hypertrophy of lenticels had been up to that time reported on conifers.

The present writers have found hypertrophied lenticels on the roots of the following conifers: *Pinus ponderosa, Pinus coulteri, Pinus rigida, Pinus resinosa, Pinus banksiana, Pinus virginiana, Pinus sylvestris, Pinus caribaea, Pinus strobus, Pinus monticola, Pinus excelsa, Picea canadensis, Picea rubens, Picea mariana,*[1] *Picea pungens, Abies balsamea,*[2] *Tsuga canadensis, Larix laricina, Taxus cuspidata, Taxus brevifolia,* and *Araucaria bidwellii*.

Several of the species of Pinus on which the hypertrophy was found were growing in the greenhouse of the United States Department of Agriculture at Washington, D. C. It was noteworthy that plants of *Juniperus virginiana* under the same conditions in the same greenhouse apparently were free from such growths so far as could be determined. In a swamp in which the hypertrophied lenticels were found on *Abies balsamea, Picea rubens,* and *Tsuga canadensis* none could be discovered on *Taxus canadensis*. Among the pines the hypertrophied lenticels were frequent mainly on the 3-needled species, *Pinus ponderosa* and *Pinus*

[1] Material furnished by Dr. H. P. Brown, of The New York State College of Forestry at Syracuse University.

[2] Dr. James R. Weir advises the writers that he has frequently found hypertrophied lenticels on the roots of *Abies grandis* in the Northwest.

rigida, while on the strictly 2-needled *Pinus virginiana*, *Pinus banksiana*, and *Pinus resinosa* they were very difficult to find. Klebahn (*10*, *p. 582*, *586*) states that up to the time of his publication he had not been able to find lenticels on *Pinus sylvestris*, nor had he satisfactorily demonstrated a substitution for lenticels.

Excrescences like those just described on the conifers are common and widespread occurrence on a number of dicotyledonous plants, particularly upon swamp plants such as *Sambucus canadensis*, *Rhus copallina*, *Decodon verticillatus*, and *Cephalanthus occidentalis*. Such excrescences on dicotyledonous plants have long been known under the term "water lenticels."

CONDITIONS UNDER WHICH HYPERTROPHY HAS BEEN FOUND

The lenticel hypertrophy observed on roots has been generally limited to plants growing in wet soil. Affected hemlock, balsam fir, red spruce, and black spruce have already been noted as growing under swamp conditions. All the pitch pines found with hypertrophied lenticels in the vicinity of Washington were in heavy, wet soil. There hypertrophy was very frequent on *Pinus rigida* and *P. virginiana* growing in swampy locations. The pines found so affected in the greenhouse at Washington were all growing in soil very much wetter than that in which they are usually found. The only Scotch pines found with hypertrophied lenticels were growing at the edge of an irrigation ditch in especially wet soil at a Michigan nursery. The same has been true in the most striking cases of hypertrophy at the Bessey Nursery. In a bed, a portion of which was repeatedly flooded from a leaking irrigation ditch, approximately 20 per cent of the plants showed marked cases of hypertrophy, while less than 1½ per cent of the plants showed hypertrophy in parts of the neighboring beds which were not affected by the leakage. Information has been received from Mr. W. H. Schrader that at the Monument Nursery of the United States Forest Service in Colorado the only conspicuous occurrence of root lenticel hypertrophy was during an unusually wet season. The hypertrophy here considered has been found both in heavy and in very sandy soils; in the latter case there was apparently more hypertrophy in parts of the beds to which clay had been added.

The youngest seedling observed with lenticel hypertrophy was one of *Pinus ponderosa* which was raised from the seed with its roots in a 2-ounce bottle of tap water in the laboratory. This water was not changed during the entire period of growth. The bottle was stoppered but was not absolutely sealed at the point of passage of the stem through the stopper. At the end of approximately five months the plant, which still seemed fairly vigorous, had developed a single root, which, after reaching the bottom of the bottle, had coiled itself around two or three times close to the peripheral limit of the bottle. On this tap root were a

number of conspicuous, glistening, mound-shaped excrescences, as is shown, slightly magnified, in Plate 46, C. A microscopic examination of sectional preparations of these excrescences (Pl. 46, A) showed clearly their lenticellular structure. The outgrowths were so loose and delicate that the outer portions were necessarily lost in sectioning, but the figure shows enough of the bases to indicate the type of structure.

In general, root-lenticel hypertrophy has been found especially frequent not only on species like western yellow pine, which are somewhat inclined to lack fine fiberous roots, but also on individuals of other species when a strong tap root has been developed with relatively little development of laterals. Whether or not the larger lenticels are of advantage to such plants in fulfilling part of the functions that the missing laterals might have performed is of course uncertain. In this connection it is of some interest to note the finding of root-lenticel hypertrophy in Michigan on white and Colorado blue spruce (*Picea canadensis* and *P. pungens*) whose roots had been injured by May beetle larvae. It is also especially interesting that nursery trees that have not been transplanted or that are in their second season in the transplant beds show decidedly less hypertrophy than recently transplanted stock. The recently transplanted trees have, of course, lost most of their absorbing roots, while the trees transplanted the preceding season have had a chance to develop normal root system again after transplanting.

IRRIGATION EXPERIMENTS

Trees of *Pinus ponderosa* in their third year in the nursery, and two months following transplanting, were given river water from the irrigating ditch frequently during a three months' period, beginning July 11, 1917. All the tests considered in this and the following section were conducted at the Bessey Nursery in cooperation with Forest Supervisor Jay Higgins and his assistants. The water added at each irrigation was approximately equivalent to 2.2 inches (5.6 cm.) of rainfall. A bed which received 31 such irrigations during these three months showed at the end of the period 31 per cent of the trees with 8 or more distinctly hypertrophied lenticels each and a total of 57 per cent with some evidence of hypertrophy. The figures are based on an examination of 255 trees. This amount of watering was sufficient to cause more or less chlorosis, especially of the shoots which arose after the watering began. Another bed in the same section, on which the frequent watering was not started until a month later and which received during the entire three months a total of 17 irrigations, showed at the end of the period eight or more enlarged lenticels each on approximately 13 per cent of the plants examined. Other beds used as controls received the usual amount of water given at this nursery, involving six irrigations in addition to the 7.7 inches (20 cm.) rainfall during the period of three months.

These showed less than 1½ per cent of the plants with abundant hypertrophied lenticels and a total of less than 13 per cent showing any evidence of hypertrophy. The results in the most heavily watered bed and in the controls are given in Table I. The results with the pruned trees shown in the table lead to the same conclusions as the results cited above on the unpruned trees—namely, that heavy watering increased the amount of lenticel hypertrophy.

TABLE I.—*Effect of watering and top pruning on root-lenticel hypertrophy of third-year western yellow pine at Bessey Nursery, Halsey, Nebr., pruned in early July and examined September 10 to 15*

Plot.	Part removed by pruning.	Number of trees examined.		Percentage of trees with hypertrophy.		Percentage of trees with strong hypertrophy.[a]	
		Heavily watered series.	Normally watered series.	Heavily watered series.	Normally watered series.	Heavily watered series.	Normally watered series.
C	All the secondary needles [b]	185	42	8	7	3	0
B	All the secondary needles [b] and tip of third season terminal shoot.	182	47	9	2	2	0
A	All the secondary needles [b] and entire third-season shoot	32	51	6	0	0	0
E	Third season terminal shoot only	108	48	41	17	19	0
D	Half the secondary needles only [b]	58	0	31	.....	17	.....
F	Unpruned	206	72	58	11	33	0
.....	Additional unpruned rows scattered among the different series	49	71	51	13	24	3
ABC	Heavily pruned	399	140	9	2.9	2.3	0
DE	Lightly pruned	166	48	37	17	18	0
.....	Unpruned	255	143	57	12	31	1.4

[a] Having 8 or more noticeably hypertrophied root lenticels per tree.
[b] Including the needles that had appeared on the third-season shoot as well as those produced in earlier years. Cut back to sheath but portion of needle remaining in the sheath left intact.

PRUNING EXPERIMENTS

Pruning experiments were conducted in an effort to throw a little more light on the factors controlling the lenticel hypertrophy. The tops of a number of rows of western yellow pine transplants at the Bessey Nursery were pruned with different degrees of severity during the first week in July, 1917. This is about the middle of the season of vigorous growth at this nursery. The results of a root examination three months later appear in Table I. The most heavily pruned plants showed the least lenticel hypertrophy, with the exception of plot E in the normally watered series. The percentage in this case is based on only 48 trees, only one-third as many as furnished the basis for each of the other figures in the three lower lines of the table. The pruning did not so injure the plants as to prevent growth entirely, for even those most heavily pruned reacted by sending out new shoots.

CAUSES OF LENTICEL HYPERTROPHY

Schenck (*15*) attributed lenticel growth on roots to oxygen hunger. However, the association which has been observed between moist conditions and abnormal lenticel growths, as well as experience in artifically producing lenticel hypertrophy by placing cuttings in water or moist air, have led more recent writers to suppose that for dicotyledonous plants the hypertrophies are directly due to the presence of an unusual amount of water (*5; 11, p. 72–80; 17*). It is reasoned, in the first place, that water or constantly moist atmosphere on the outside of the lenticels allows the steady growth of the lenticels, while dry or intermittently dry air tends to dry out the superficial cells of the lenticels or to increase their solute concentration, with resultant chemical changes, including cork and lignin formation. According to this idea the growth of the lenticel tissue is controlled by transpiration through the lenticels; with intense transpiration the tissues become dried and the hypertrophy is checked. The suberized or lignified layers thus formed are supposed to restrain mechanically further proliferation on the part of the cells beneath them from which the lenticel structures arise. So far this supposition seems logical, though there is as yet no basis for a quantitative estimation of the importance of tissue drying in the phenomenon.

DeVaux has advanced another theory, based on the fact that the supplying of abundant water to the absorbing surfaces and the reduction of transpiration have both been found to be followed by lenticel hypertrophy in experiments with dicotyledons. This writer supposes that both these treatments result in increased sap pressure in the plant as a whole and exert their influence entirely through increased sap pressure. He does not apparently give sufficient weight to the possibility that both decrease in transpiring surface and increase in soil moisture may involve decreased oxygen supply as well as increased sap pressure. The limited aeration of wet soils is a matter on which there is general agreement. The necessity of soil oxygen for the normal development of mesophytic plants, as indicated by common observation, has been recently confirmed by direct experiments by Cannon and Free (*3*) and by Livingston and Free (*12*). It is by no means certain that over-wet soil results in increased sap pressure in mesophytic plants, especially since the last-named authors find that a deficiency of oxygen in the soil results in some cases in decreased water absorption. The association between swampy soil and lenticel hypertrophy is at least as easily explained on the basis of oxygen hunger as by DeVaux's "hyperhydrose" doctrine.

The argument which Tubeuf (*20, 21*) seems to consider strongest against oxygen hunger as the stimulus for lentical enlargement is the fact that enlargement can be produced in cuttings in a moist chamber. By placing cuttings with paraffined ends in moist chambers he secured lenticel overgrowth, even in cases in which an atmosphere of oxygen was

provided. This seems at first glance to dispose of the oxygen-hunger hypothesis quite effectively. However, an atmosphere of oxygen would not necessarily insure an oxygen supply to the interior of a woody stem unless the lenticels were already open at the time the cutting was placed in the chamber. A section of stem removed from the plant and therefore deprived of the oxygen that it would normally get from the leaves and perhaps also from the roots, if its lenticels were closed, might easily by oxidation of stored food materials develop abnormal partial pressures of carbon dioxid in its interior tissues which would not be relieved till the lenticels were opened by the stimulated growth which Tubeuf describes. The experience reported in his later paper, in which he records interesting cases of lenticel stimulation secured by covering bark with impervious materials, and observation of lenticel hypertrophy on the swelling above a heat lesion lead him to consider the stimulation lenticel growth too complicated to be explained by any single factor so simple as humidity. He still appears to consider oxygen hunger as excluded from further consideration. However, his observation of numerous lenticels on the stem of a heart-rotted spruce is the only reference that has been found concerning abnormal lenticel growth on any part of a coniferous tree.

The intumescences produced by Atkinson on tomato (*1*) and by Douglas on potato (*6*) were clearly related in some way to excessive general sap pressure. They are not analogous cases to the root lenticels here considered, since the hypertrophy in the intumescences was, so far as can be judged from the illustrations given, mainly due to the stretching of soft tissue cells already present rather than to the formation of large masses of new cells.

It may be of some interest to note in passing that Cowles (*4, p. 553-554*) expresses himself as inclined to regard lacunar tissue in submerged parts of water plants to be a response to lack of transpiration rather than to oxygen deficiency.

The present writers' findings bearing on the factors causing hypertrophy of subterranean lenticels on young conifers are as follows:

1. Hypertrophy is apparently limited to trees with their roots in water or very wet soil. This may indicate either increased sap pressure or decreased aeration as among the effective stimuli. It seems rather improbable that there should be a significantly greater sap pressure in a mesophyte like *Pinus rigida* or a semixerophyte like *P. ponderosa* (Rocky Mountain type) in an excessively wet soil than in a plant in more normal condition. This seems especially improbable in view of the slow water absorption by the mesophytes in soil deficient in oxygen in the experiments already referred to (*12*).

2. While lenticel hypertrophy seems to be most common in soils containing clay, it has been frequently found in one nursery (at Halsey,

Nebr.) having a very sandy, well-drained soil, with a wilting coefficient[1] in the neighborhood of 3.4 per cent for the nursery as a whole, and an unusually high proportion of the soil (79 per cent) in particles between 0.25 and 0.05 mm. in diameter. The results of a mechanical analysis of this soil have already been published (*8*, *p. 2*). This, at first thought, indicates sap pressure rather than deficient aeration as the cause of hypertrophy. It is worthy of note, however, that in this case there was frequent artificial watering in addition to considerable rainfall, and it is therefore entirely possible that even in this case aeration was insufficient. Buckingham (*2*) found that both diffusion and molar movement of gas were slower in a wet sand than in any of the other soils, wet or dry, with which he experimented.

3. Reduction of the transpiring surface by removal of a large part of the needles, or of the terminal growth, or both, resulted in distinctly reducing the tendency to lenticel hypertrophy. (Table I.) The unpruned plants presumably had, at least part of the time, a lower general sap pressure than the pruned. The result of the experiment therefore tends to diminish the probability that there is any important causal relation between general excessive sap pressure and the hypertrophy in question.

4. The finding of the most abundant hypertrophy on roots which are deficient in fibrous laterals or whose absorbing surface has been greatly reduced by insect injury or by transplanting also tends to weaken the hypothesis that excessive general sap pressure throughout the plant is the chief cause of the hypertrophy. It is possible that roots which have little absorbing surface will take less oxygen from the soil than would better-developed root systems. An indication that this is the case is seen in the experience of Livingston and Free (*12*, *p. 185*) with the oxygen requirements of roots with different amounts of surface area. This association between deficient root surface and lenticel hypertrophy may therefore be an indication of a relation between oxygen deficiency and lenticel production.

The fact that lenticel hypertrophy was actually less in plants whose leaf surfaces had been reduced by pruning not only tends to decrease the probability of the "hyperhydrose" explanation; it is suggested that it is perhaps a further support for an oxygen-hunger (or carbon-dioxid excess) hypothesis. Plants with their leaf surfaces reduced during the latter part of the summer will of necessity produce less carbohydrate. The smaller amount of carbohydrate reaching the roots in consequence of the pruning might conceivably result in less respiration in the root tissues and therefore in a decreased need for oxygen. If this were the case the decreased oxygen hunger might furnish a partial explanation of the slight lenticel growth in the pruned plants.

[1] Determined by the Office of Biophysical Investigations, Bureau of Plant Industry.

Another possible connection between leaf pruning and oxygen hunger of root and stem is suggested by Prof. Livingston in a personal communication to one of the writers. A reduction of the transpiring surface by pruning should result in less absorption by the roots. If it be supposed that oxygen dissolved in water absorbed from the soil is important as a source of oxygen supply for the root tissues, a decrease in the amount of water absorbed might result in oxygen deficiency in the root tissues. This suggestion might help to explain the earlier reports of the stimulated growth of lenticels on stems of dicotyledons whose transpiration has been experimentally reduced. It obviously complicates any attempt to explain on an oxygen-hunger basis the effects of pruning on lenticel growth described in the present paper.

Of course it does not seem likely that any part of a plant accustomed to the presence of free oxygen would be likely to make much growth in the entire absence of oxygen. However, the condition existing in the soil in which the hypertrophies occurred certainly did not involve the entire absence of oxygen. Pfeffer concludes (*14*, *p.* *115*), in spite of some conflicting evidence, that experiments have shown that reduction of the proportion of oxygen, at least in some cases, acts as an accelerating stimulus to growth.

It is of course true that any strong local growth is probably dependent on high local sap pressure. However, it is well known that such local high pressures are not necessarily dependent on excessive turgidity of the plant as a whole. Unusual chemical conditions, such as might conceivably result from local oxygen hunger, might easily cause them. The writers do not consider that oxygen hunger is established as the main cause of the lenticel hypertrophy found. They can not, however, agree with De Vaux in attributing the effect of increased soil moisture on lenticel growth entirely to increased water supply, excluding oxygen hunger as a possible factor in stimulating lenticel growth.

Experiments in which the oxygen, carbon-dioxid, and water supplies in the soil are independently controlled, as by the technic of Livingston and Free (*12*), and perhaps also with temperature control, will be needed to make a beginning on determining the relative importance of these various environmental factors in causing hypertrophy of root lenticels. Since conifers are rather difficult to handle in experimental work, poplar would perhaps be a better subject for preliminary experimentation. It seems likely, as has been suggested for hypertrophied lenticels in general by Tubeuf (*21*) and for intumescences by Hasselbring (*9*), that these unusual lenticel enlargements on the roots of conifers depend on a complex of conditions rather than on any one simple stimulus, and that with different species the conditions which call forth lenticel hypertrophy may be found to differ in relative importance.

RELATION BETWEEN LENTICEL HYPERTROPHY AND HEALTH OF PLANTS

Sorauer (*17, p. 210–219*) has used the name "tan disease" for lenticel hypertrophy on roots and stems of fruit trees. His use of the term "disease" appears justified in view of the association in many cases between the lenticel hypertrophy and a general pathological condition of the trees. The large lenticels described in the foregoing paragraphs as occurring on conifers are undoubtedly abnormal and in that sense are pathological. Since they occur only in abnormally wet situations, it is to be expected that in many cases the pines on which they have been found are unused to very moist surroundings and under the unfavorable conditions are subnormal in general vigor. The hypertrophies were first noted in a part of a nursery in which general vigor was unsatisfactory. Comparisons of the less vigorous and more vigorous plants in the section in which the hypertrophy was common showed lenticel hypertrophy present in both the weaker and stronger plants. The first examination, made by Hartley on about 200 3-year-old transplants of *Pinus ponderosa*, showed lenticel hypertrophy on a larger proportion of the weak trees than of the stronger trees. Later examinations made by Hahn on about 2,000 plants showed, particularly on *P. ponderosa*, that the greatest number of hypertrophied lenticels were associated with vigorous growth, This was true of plants in which the terminal root was rapidly advancing and the roots were large and stocky but correspondingly undeveloped as to lateral root surface. In one particular instance, however, where 2-year-old transplants of *P. ponderosa* had been badly affected by yellowing due to excessive irrigation, 50 per cent of 95 vigorous plants examined showed light occurrence of lenticel formation, while of 110 weakened and dying plants 80 per cent were found to exhibit light occurrence, and 10 per cent showed pronounced occurrence. This same bed examined a month later showed that the majority of the weak plants had died, while the vigorous plants, or those beginning to show renewed terminal growth, were alone showing freshly proliferating lenticels, those upon the dying plants becoming darkened and sloughing off. It therefore appears that lenticel hypertrophy is found on both weak and strong plants and that the conditions which bring on their formation may, if sufficiently prolonged, eventually cause the weakening and death of the plant. There is, however, so little direct connection between lenticel hypertrophy and the pathology of the conifers that it seems logical to recommend that any further investigation of the factors stimulating lenticel growth should be made from the point of view of physiology rather than from that of pathology.

SUMMARY

(1) Unusual excrescences on the roots of a number of different pines, spruces, and other conifers are found to have the structure of lenticels, much enlarged. They are produced in various kinds of soil in the presence of excessive moisture. Hypertrophy may occur on either weak or vigorous plants. Hypertrophy was decreased by top pruning and was increased by root injury. Such overgrowths have apparently not been previously reported on conifers.

(2) Conclusions of certain writers, based on work with dicotyledons, that excessive soil moisture stimulates lenticel hypertrophy mainly by increasing general sap pressure and that oxygen hunger is of no importance as a stimulus are not supported by the experience here set forth with conifers. Experiments in which the oxygen supply to the roots is varied without varying the water supply are believed necessary to settle the relative importance of these two factors.

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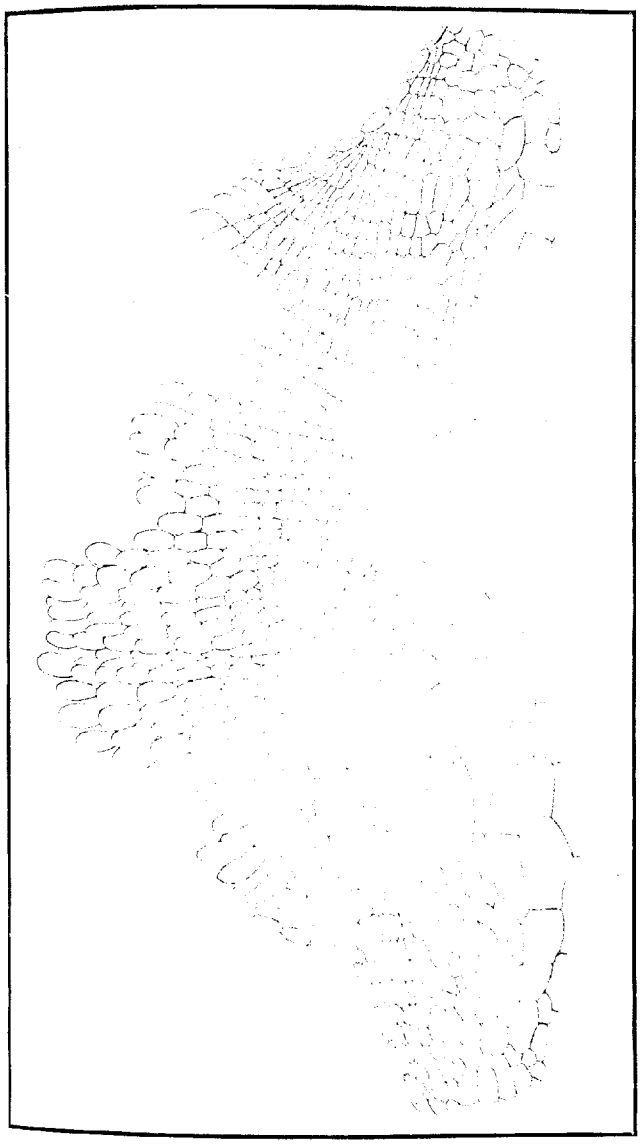
PLATE 44

Section through a hypertrophied lenticel on root of *Pinus rigida* growing in swampy situation. Approximately × 59.



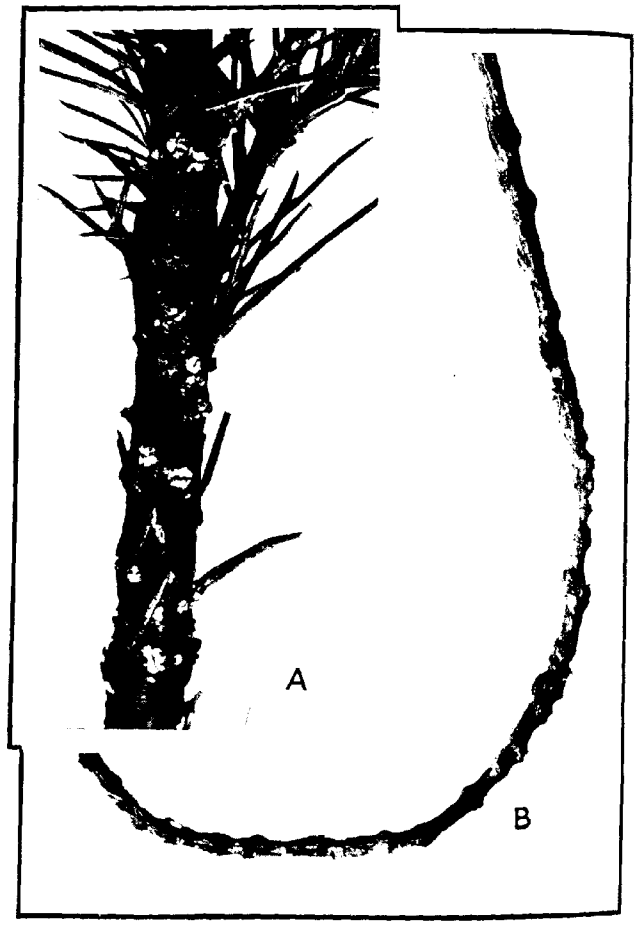

A
B

PLATE 45

A.—Hypertrophied lenticels on the basal part of layering stem of *Picea mariana*, which had been covered with sphagnum. Approximately × 1¾.

B.—Tap root of a *Pinus ponderosa* transplant, bearing an unusually large number of hypertrophied lenticels. Approximately × 1¾.

PLATE 46

A.—Cross section of the stem through one of the hypertrophied lenticels shown in C. In embedding and sectioning most of the loose outer tissues are unavoidably lost. Approximately × 112.

B.—Large patches of excrescences upon the tap root near the root crown, on *Pinus rigida*. Approximately × 1¾.

C.—Hypertrophied lenticels on root of 5-months-old *Pinus ponderosa*, grown in a loosely stoppered 2-ounce bottle, in tap water which had not been changed since the germination of the seed. The entire structure of the lenticel, which is too delicate to recover in digging roots from the soil, is here preserved. Approximately × 1¾.


A
B
C

DEGREE OF TEMPERATURE TO WHICH SOILS CAN BE COOLED WITHOUT FREEZING

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The general impression seems to be that when the temperature of soils falls slightly below the freezing point (0° C. or 32° F.) they freeze, that is, the soil moisture is converted into ice. This is hardly the case, however. In conducting investigations to study and measure the different forms of water in the soil by means of the dilatometer method[1] and to study and measure the concentration of the soil solution directly in the soil by means of the freezing-point method,[2] it was discovered that it is almost impossible to freeze the soils when they are cooled only slightly below the freezing point. This is true even when the concentration of the soil solution is exceedingly small and the freezing-point depression consequently negligible. Indeed, it was found that it is difficult to start solidification in the soils unless they are supercooled at about 1° C. below their true freezing point. Even at this degree of undercooling freezing begins only with vigorous agitation. If the soil is not vigorously agitated or disturbed it will remain at this temperature indefinitely without freezing. As the degree of undercooling is increased, however, the ease with which solidification is induced is also increased. Finally a temperature is reached where freezing starts automatically without agitation of the soil mass. This critical temperature is surprisingly low for all soils, as will be observed from the experimental data presented in Table I. This table shows the amount of cooling which the soils are able to withstand without freezing. The procedure by which these experimental results were obtained consisted in placing a 1-inch column of wet soil in a freezing-point tube, inserting the bulb of a Beckmann thermometer into this column of soil, and cooling the soil in different low temperatures until a temperature was reached where freezing would readily take place automatically. The figures represent approximately the limit of supercooling which these soils can resist without freezing. At this maximum degree of supercooling the soils can be maintained indefinitely if they are not disturbed or agitated. With a slight disturbance or agitation, however, they will

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readily freeze. Again, if the temperature of cooling is only slightly lowered the soils will immediately freeze. These numerical data, therefore, represent just about the maximum cooling which the soils can withstand indefinitely without freezing when they are kept quiet.

For the sake of an interesting comparison, Table I also presents the limit of supercooling without freezing of several artificial materials.

TABLE I.—*The degree of cooling which soils and artificial materials can withstand without freezing when they are kept quiet and with the water content at about the saturation point*

Material.	Degree of supercooling without freezing.
	°C.
Quartz sand	−4.2
Coarse sand	−4.2
Fine sand	−4.2
Very fine sand	−4.2
Stony loam	−4.2
Loam	−4.2
Silt loam	−4.2
Clay loam	−4.2
Humus loam	−4.2
Clay	−4.2
Red clay	−4.2
Dark clay	−4.2
Brick clay	−4.2
Clay subsoil	−4.2
Peat	−5.0
Muck	−5.0
Water	−6.0
Silica	−6.0
Carbon black	−6.0
Gelatin	−6.0
Agar	−6.0

An examination of the foregoing experimental results reveals at once the fact that the amount of cooling which the soils are able to withstand without freezing is considerable, being about −4.2° C. (7.56°F.) for the mineral soils and about −5° C. (9° F.) for the peats and mucks.

It is of interest to observe that the maximum supercooling is still greater for the water and for the artificial materials, amounting in all cases to about −6° C. (10.8° F.). Since water freezes at about the same degree of supercooling as the artificial materials, it would logically seem that it is the water which limits the degree of supercooling of those materials and that they themselves have no influence on the degree of supercooling of water in one way or the other.

The question now rises, why do the soils withstand a smaller degree of supercooling than the artificial materials?

No definite explanation can be offered for this phenomenon. It would appear, however, that the true explanation is to be found in the difference in the size of particles of the two classes of materials. The artificial materials possess, of course, incomparably finer-sized particles than the soils do, and it would seem that when the division of a substance approaches the molecular state it ought not to affect the freezing of water materially. However, in a series of experiments conducted to ascertain if clay soils could withstand a greater degree of supercooling than coarse sands, it was found that sands with infinitely larger-sized particles resisted freezing equally as well as clays. It is possible, therefore, that other factors, such as the nature of the material, its cohesive and adhesive properties, its specific gravity, etc., also come into play in affecting the degree of supercooling.

In order to ascertain if the degree of moisture content exerts any influence upon the resistance of soils to freezing, different water contents were employed in all the various soils. The results failed to show, however, that moisture had any appreciable influence on the resistance of soils to freezing. Soils at a very low moisture content could not be supercooled any further than at a very high moisture content.

The foregoing experimental results afford a new and significant insight into the temperature of soils during the cold seasons. In the first place, they go to show that mineral soils may be cooled down to −4.2° C. (7.54° F.) and peats and mucks down to −5° C. (9° F.) without freezing. This being the case, the conclusion naturally follows that during mild winters and in mild climates in the winter the soils may not freeze even though they are cooled below their freezing point.

In the second place these findings prove quite conclusively that the method now in vogue for measuring temperature in soils in cold seasons may not give entirely the true facts. The thermometers will be recording the temperature to be several degrees below the freezing point and yet the soils may not be actually frozen.

The foregoing experimental results are very significant from still another standpoint. As it is well known, water in the liquid state has twice the specific heat that ice has. As long as the soil moisture remains in the liquid state the temperature fluctuations in the soil will be correspondingly slower and smaller.

Indeed, the ability of soils to resist freezing even when their temperature is much below the freezing point throws considerable new light on questions regarding the temperature of soils in cold seasons and consequently upon the physical, chemical, and bacteriological processes going on in the soils during those seasons.

CHANGES TAKING PLACE IN THE TEMPERING OF WHEAT

By E. L. TAGUE

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In milling wheat it has been found advisable to "temper," "dampen," or "condition" the grain before grinding. This process consists in adding a certain amount of water to the wheat, then thoroughly mixing and allowing it to stand for a time. The treatment toughens the bran coat of the kernel, thus making possible a closer separation of the bran and the flour, and increases the desirable milling qualities of the wheat in other ways. The yield of flour is increased, and a flour is obtained from which better bread can be made. All practical millers are well acquainted with the fact that tempering improves the milling quality.

That Jago [1] recognizes the fact is shown by the following quotation:

> On making baking tests with the flours from such slightly dampened wheats, compared with those of the wheats milled dry, the dampened wheat flours fall off less during fermentation, yield bread of a better color and flavor, and in practically the same quantity. The slight damping of very dry wheats enables the miller to produce a better quality of flour.

Swanson [2] observes that conditioning not only toughens the bran of the wheat and makes it easier to crush the endosperm but it also affects the quality of the gluten and the baking quality of the flour. Temperature, moisture, and time play an important part in this process. Improvement through conditioning is similar to that brought about by natural ageing.

The changes in the flour are probably either physical or chemical, or more likely a combination of the two. The thorough elimination of the bran gives a flour of better color, and the closer separation of the bran and the endosperm produces a flour of higher gluten content. It is possible that the quality of the gluten is also affected. If so, this would indicate a chemical change during tempering or a physical change of such a nature as to make possible a more pronounced chemical change during fermentation and baking.

Since the experience of practical millers indicates that the physical changes mentioned above do occur, the subject is one which calls for accurate investigation. Millers often ask the question whether the obvious physical changes are accompanied by chemical changes. If so, a standardization of the factors which govern the tempering of wheat would lead to a more uniform product.

[1] JAGO, William, and JAGO, William C. TECHNOLOGY OF BREAD MAKING, p. 360. London, 1911.
[2] SWANSON, C. O. WHEAT CONDITIONING. *In* Amer. Miller, v. 41, no. 6, p. 467-469, illus.

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The principal factors involved in the tempering of wheat are (1) time, (2) amount of water added, and (3) temperature. These factors vary somewhat with different varieties of wheat. The general practice of millers seems to be to temper from 12 to 48 hours and to add sufficient water to make the total moisture content 15½ per cent. There does not seem to be any fixed temperature used. Some millers pay no attention at all to this factor, while others "warm" the water before adding it to the wheat.

EXPERIMENTAL WORK

Three varieties or lots of wheat were used for the experimental work—a variety of hard wheat known as Kanred, developed recently by the Kansas Agricultural Experiment Station; a hard, red wheat (Turkey or Kharkof) from central Kansas; and a soft wheat from Colorado. This latter variety came to the department as Arizona White wheat.

The only chemical changes considered in this study were changes in the (1) hydrogen-ion concentration, (2) total acidity, (3) water-soluble phosphorus, and (4) titrable nitrogen. Yields of straight flour were also computed, and the milling qualities were judged as nearly as possible. Other investigations under way at the present time will be reported in a later paper.

Preliminary experiments were first conducted, from the results of which it seemed advisable to compare different periods of time, different temperatures, and different moisture contents as follows: (1) Time, 24 hours, 48 hours, and 72 hours; (2) temperature, 5°, 20°, and 40° C.; and (3) moisture content, 15½ and 18 per cent. The preliminary experiments seemed to indicate that the best results would be secured within these limits.

APPARATUS AND METHODS

The wheat was ground in a small burr mill driven by an electric motor. This mill was so made that it could be taken apart easily and cleaned. In addition, it was fitted with bran and flour sieves of silk bolting cloth.

The wheat was tempered and extracted in a large water thermostat fitted with a stirring device run by a small water motor. The thermostat was heated by a gas burner, and the temperature was kept constant (within 1° C.) by means of a mercury gas regulator.

The same hydrogen-ion apparatus was used as that described in a former paper,[1] excepting that the saturated potassium-chlorid electrode was used instead of the normal potassium-chlorid electrode.

The original moisture content of each lot of wheat was determined by drying in the air oven at 110° C. to constant weight. This was found to be 12.65 per cent for Kanred, 10.86 per cent for the Hard Red winter wheat, and 10.80 per cent for the Arizona White. In preparing the wheat and flour samples 200 gm. of wheat were weighed out into a 500-cc. bottle. To this was added sufficient distilled water to bring

the total moisture content up to the desired percentage. The bottle was corked tightly and the mixture was well shaken. The bottle was then placed in the thermostat, which had been brought to the desired temperature, and the mixture was allowed to remain in the thermostat for the desired length of time. At the end of the time the wheat was ground as rapidly as possible in the mill. The mill was set to grind to the same fineness for each lot of wheat, and each lot was put through the mill the same number of times. During the grinding the milling qualities were judged as nearly as possible, and after grinding the yields of straight flour were calculated.

Sufficient flour to equal 50 gm. on a moisture-free basis was immediately weighed out. This was placed in a fruit jar, and sufficient distilled carbon-dioxid-free water was added to make the ratio of moisture-free flour to water 1 to 10. This water had been previously heated to 40° C. To this mixture 2 cc. of toluene were added as a preservative, the jar was tightly closed by means of a rubber and a screw cap, and the contents were thoroughly mixed by shaking. The jar was then placed in the thermostat, where the temperature was 40° C. The flour was extracted for 2 hours at this temperature, the jar being well shaken every 15 minutes. At the end of 2 hours the jar was removed and the contents were poured into a centrifuge cup. The cup was then placed in the centrifuge and whirled for 5 minutes at a speed of 2,500 revolutions per minute. Finally the supernatant liquid was poured through a folded filter, and the filtrate was used for the determinations of hydrogen-ion concentration, total acidity, water-soluble phosphorus, and titrable nitrogen. For the determination of the hydrogen-ion concentration and total acidity 100 cc. of the filtrate were pipetted into an electrode vessel. The vessel was then placed in the hydrogen-ion apparatus, and hydrogen gas was passed through until the potential remained constant (within 1 millivolt) for 15 minutes. During the entire time the vessel was shaken 60 times per minute. After this constant potential was noted, *N/10* alkali was run in from a burette in small portions at a time until the constant potential indicated a P_H value of 7, which is the absolute neutral point. The number of cubic centimeters of *N/10* alkali used were then taken to represent the total acidity.[1]

The water-soluble phosphorus was determined from a second 100-cc. portion from the same filtrate. The phosphorus was determined by the usual method after the organic matter had been destroyed by boiling with nitric acid. The titrable nitrogen was determined in a third 100-cc. portion by the formaldehyde method of Sorensen, using thymolphthalein as an indicator. The number of cubic centimeters given in Table I multiplied by 1.4 gives the number of milligrams of titrable nitrogen in 100 cc. of the extract.

For a control, a portion of each variety of wheat, untempered, was ground, and an extract was made of each in exactly the way described

[1] For fuller description see SWANSON, C. O., and TAGUE, E. L. op. cit.

above. The same determinations were then made on these extracts as on the tempered lots.

The results for each variety of wheat are presented in Tables I, II, and III.

TABLE I.—*Yield of flour, hydrogen-ion concentration, total acidity, water-soluble phosphorus, and titrable nitrogen of the flour from Kanred wheat*

Time tempered.	Temperature.	Hydrogen-ion concentration.	Total acidity.[a]	Water-soluble phosphorus.	Titrable nitrogen.[a]	Yield of flour.[b]	Remarks.
Hours.	°C.	P_H		*Per cent.*			
..	.....	6.20	1.7	0.0361	4.2	68	Brittle and hard.
24	5	6.20	1.7	.0359	4.2	67	Ground fairly well.
48	5	6.22	1.8	.0360	4.1	69	Do.
72	5	6.20	1.7	.0361	4.3	68	Do.
24	20	6.17	1.7	.0361	4.4	70	Do.
48	20	6.19	1.7	.0362	4.6	72	Ground well.
72	20	6.13	1.8	.0380	4.5	71	Do.
24	40	6.06	2.0	.0376	4.6	72	Do.
48	40	6.00	2.0	.0379	4.6	72	Do.
72	40	6.00	2.0	.0378	4.8	70	Sticky.

[a] Expressed as number of cubic centimeters *N*/10 sodium hydroxid required to titrate 10 gm. flour.
[b] Expressed as number of grams of flour obtained from 100 gm. of wheat.

TABLE II.—*Yield of flour, hydrogen-ion concentration, total acidity, water-soluble phosphorus, and titrable nitrogen of the flour from Hard Red winter wheat (Turkey or Khorkoj)*

Time tempered.	Temperature.	Hydrogen-ion concentration.	Total acidity.[a]	Water-soluble phosphorus.	Titrable nitrogen.[a]	Yield of flour.[b]	Remarks.
Hours.	°C.	P_H		*Per cent.*			
.....	.....	6.13	1.9	0.0469	3.6	65	Brittle and hard.
24	5	6.13	1.8	.0460	3.8	67	Somewhat softer.
48	5	6.15	1.9	.0468	3.7	67	Do.
72	5	6.10	1.9	.0472	3.8	68	Do.
24	20	6.06	1.9	.0472	3.9	68	Ground fairly well.
48	20	6.06	1.9	.0471	3.8	69	Do.
72	20	6.06	1.9	.0476	4.0	70	Ground well.
24	40	5.92	2.2	.0479	4.0	71	Do.
48	40	5.91	2.1	.0483	4.1	70	Do.
72	40	5.92	2.2	.0482	4.0	68	Sticky.

[a] Expressed as number of cubic centimeters of *N*/10 sodium hydroxid required to titrate 10 gm. flour.
[b] Expressed as number of grams of flour obtained from 100 gm. of wheat.

TABLE III.—*Yield of flour, hydrogen-ion concentration, total acidity, water-soluble phosphorus, and titrable nitrogen of the flour from Arizona White wheat*

Time tempered.	Temperature.	Hydrogen-ion concentration.	Total acidity.[a]	Water-soluble phosphorus.	Titrable nitrogen.[a]	Yield of flour.[b]	Remarks.
Hours.	°C.	P_H		*Per cent.*			
.....	.....	5.89	1.5	0.0177	2.0	67	Brittle.
24	5	5.92	1.5	.0178	1.9	67	Ground well.
48	5	5.92	1.5	.0176	1.9	68	Do.
72	5	5.89	1.5	.0179	2.1	68	Do.
24	20	5.89	1.6	.0179	2.1	70	Do.
48	20	5.86	1.6	.0181	2.2	72	Do.
72	20	5.86	1.7	.0182	2.1	70	Do.
24	40	5.82	1.7	.0186	2.3	71	Do.
48	40	5.82	1.8	.0185	2.3	70	Sticky.
72	40	5.79	1.8	.0184	2.1	69	Do.

[a] ... titrate 10 gm. flour.

The addition of sufficient water to make the total moisture content 18 per cent was tried with each variety of wheat. In every case the resulting flour was sticky, the sieves became clogged, and the yields were reduced below that for the untempered grain. For this reason the analyses of the flour from this treatment were not completed.

It will be noted that when the wheat was tempered at 5° C. there was practically no chemical change as compared with the untempered wheat. As a general rule the yields were slightly higher and the milling qualities were considerably better than those secured from the control or untempered wheat. In each case the bran was tougher, and a cleaner separation of the bran and endosperm was possible. The length of time appeared to have very little influence on either the physical or chemical composition of the flour.

When the wheat was tempered at 20° C., a small but definite chemical change took place. The hydrogen-ion concentration was increased, as was shown by a lower P_H value. The total acidity, the water-soluble phosphorus, and the titrable nitrogen were also higher. Both the yield and the milling quality were better than when the wheat was tempered at 5° C. The time of tempering appeared to be a factor in the chemical changes but had very little if any relation to the physical qualities.

The chemical changes were still more pronounced when the grain was tempered at 40° C. The physical changes appeared to be detrimental to the milling qualities of the grain. In other words, increasing the time of tempering increased the chemical changes but proved detrimental after 48 hours so far as the milling value of the wheat was concerned.

In general the milling qualities of the drier wheats were improved by tempering more than were those of the wetter wheats, and the hard wheats were improved more than the soft wheats.

It may be concluded from these experiments that slight chemical changes take place during the tempering process and that these changes increase with time and temperature. Improvement in the milling qualities is confirmed also, excepting in cases where the time of tempering exceeded 48 hours and where the temperature exceeded 20° C. It would appear from this that (1) the improved milling quality of tempered wheat is due chiefly to physical changes, (2) a temperature of 20 to 25° C. is best, (3) 15½ per cent moisture appears to be about the best, (4) the maximum improvement takes place in 48 hours, (5) hard wheats are improved more than soft wheats, and (6) dry wheats are improved more than wet wheats.

VASCULAR DISCOLORATION OF IRISH POTATO TUBERS

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INTRODUCTION

The exact significance of vascular discoloration in the stem-end tissues of Irish potato tubers has never been fully determined. Various types of both flesh and vascular necrosis are recognized, some of which are associated with the presence of Fusaria of various species or with *Verticillium albo-atrum*. Others, however, at least in the initial stages, yield no organisms when subjected to culture, nor does the microscope reveal the presence of organisms. It is also recognized that a superficial necrosis may develop in the stem tissues of apparently perfectly normal stock. There is no such perfect natural abscission of the potato tuber from the stolon as is common with fruits. Moreover, they are frequently harvested before the plants are mature, and the tubers are then broken off from green stolons. It has been assumed that suberization of the wound thus made normally follows in two or three days, so that not more than a few layers of dead cells should appear unless some aggressive parasite gains entrance to the wound. A popular impression has prevailed that any except the most superficial stem-end discoloration might be taken as a trustworthy indication of the presence of Fusarium, or, at least, that the stock was grown on vines affected with Fusarium or Verticillium.

Somewhat extensive preliminary observations and cultural studies, made by the writer both at the time of harvest and during or at the close of the rest period, on stock grown in sections where Fusarium blight and wilt do not occur, as well as in sections where they are known to be general, show that, while Fusarium and Verticillium undoubtedly do cause vascular discoloration of potato tubers, discoloration can not be accepted as proof of the presence of Fusarium or, indeed, of any other organism, nor can the absence of discoloration be confidently accepted as proof of the sterility of the vessels near the stolon attachment. There seems to be reason to think that vascular necrosis may often arise from purely physiological causes and that it need not necessarily be seriously abnormal, though frequently it is. A more complete discussion of this question must await the outcome of studies at present incomplete, but it seems advisable to present some available data regarding the fungous flora of potato stem ends.

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The notes from which these data have been compiled were obtained jointly by Venus W. Pool, M. B. McKay, H. G. MacMillan, R. D. Rands, and the writer during the spring and summer of 1915. The writer wishes to make full acknowledgment to these associates and to assume the entire responsibility for the construction placed on the notes and the deductions made from them, as well as for the accuracy of the tabulations and compilations presented.

OUTLINE OF METHODS AND WORK

The general plan followed in the work may be outlined briefly as follows: Material for experimental plantings, involving about 4 acres of plots, was secured from various sources, as reported below. It was treated 30 minutes in 1 to 1,000 mercuric chlorid solution and allowed to dry, after which each tuber was examined for vascular discoloration by removing with a flamed and cooled scalpel a shallow cone of tissue with the stolon attachment at the center of its base. A record was made of the presence or absence of discoloration and of the general character of the discoloration when present, as slight, medium, brown, dark, etc. When discoloration was found, the depth to which it penetrated in the tuber was determined by removing a wedge of tissue. When browning was confined to a shallow area around the removed cone it was designated by recording the symptom A. If the discoloration extended to a greater depth, involving up to one-fourth the length of the tuber, symptom B was recorded. A deeper discoloration was designated by C. Discolored tubers were submitted to culture. In general one planting of tissue was made from each region involved in discoloration. As a rule, therefore, one planting was made from tubers showing symptom A, two from those showing symptom B, three from tubers showing symptom C, and none from those showing no discoloration. In the actual prosecution of the work, however, certain deviations from the general rule were introduced, either to check the dependability of results or to secure additional information. The tubers of each lot were weighed and numbered consecutively in the order of their respective weights, which were recorded. With the exception of lot No. 3, the tubers of each lot weighing less than 3 ounces were divided into two groups, one comprising all the even numbers and the other all the odd numbers. Those weighing 3 ounces or more were halved from stem to apex, one half being placed with the small tubers of even number and the other half with the small tubers of odd number. When the half tubers weighed 3 ounces or more they were cut into stem and apex portions. In a few cases the half tubers were so large as to yield stem, middle, and apex pieces, or even stem, two middle, and apex pieces—four in all from each half. The minimum seed piece for cut tubers was 1½ ounces.

The two lots of seed stock were planted and grown in widely separated regions and under distinctly different environmental conditions of soil and climate, one lot being planted on a light, sandy soil, under rainfall, at Waupaca, Wis., and the other on a heavy clay loam under irrigation at Greeley, Colo. The identity of each plant was preserved, and frequent records were made by the same observers in rotation in each and in both regions to secure all the data possible regarding the influence of the seed piece and environment and of the interrelations of these upon individual plant performance, with special reference to the development of pathological conditions.

DESCRIPTION OF MATERIAL

The material may be divided advantageously for consideration into three groups, each containing several lots. The first group comprises stock affected with tuber-borne diseases of undertermined origin; the second lot is from healthy parentage; and the third is from diseased parentage where the malady is regarded as of parasitic origin. For brevity in presentation many lots which were held separate during the investigation have been combined, so as to appear as a unit, whenever their origin and performance made such treatment feasible.

A brief description and index of the lots presented in the tables follows.

A.—OBSCURE DISEASE GROUP.

1. Thirty-four seedling varieties originated by Prof. Wm. Stuart, of the Department of Agriculture, and originally regarded as promising but ultimately discarded because of the persistent reappearance of destructive but imperfectly understood hereditary diseases. This material had been grown at Jerome, Idaho, in 1913 and 1914, in the pathological plots there.

2. The progeny of 31 hills of Western Peach Blow, grown at Greeley, Colo., which were suspected of Fusarium infection. This stock is now known to be affected also with leafroll and mosaic and is therefore placed in this group.

3. A miscellaneous collection of 21 lots from the pathological collection of the field station at Presque Isle, Me. Both seedling and commercial varieties affected with leafroll, mosaic, and dwarfing diseases were included. This lot was grown only at Greeley, Colo., and the tubers were either planted whole, or, if they weighed over 3 ounces, they were cut once crosswise into stem and apex halves.

B.—HEALTHY GROUP.

4. A representative commercial lot of the variety Late Ohio, grown at Greeley, Colo., in 1914 and obtained from the grower.

5. An exceptionally good commercial strain of the variety Pearl, grown in Greeley, Colo., in 1914, obtained from the grower and collected from the field at harvest time.

6. A fine commercial strain of the variety Pearl, grown at Crandon, Wis., in 1914 and reported to be free from wilt, leafroll, and similar diseases.

7. Wisconsin certified seed potatoes, variety Pearl, secured from the grower.

8. Culls from two lots of Maine-grown stock of the variety Pearl. One of these lots was reported healthy and the other as diseased with leafroll. There was no difference in the performance of the two lots in either locality where they were grown, and disease was absent. They are therefore grouped together as healthy.

9. Certified seed potatoes of the variety Rural New Yorker, grown at Boss Lake, Wis. A second lot of similar, though uncertified, material of the same variety but from another grower near Racine, Wis.

10. A small lot of Wisconsin-grown stock of the variety Pearl, composed of tubers on the stolons of which Colletotrichum pycnidia were developing.

11. Four so-called types of commercial stock of the variety Rural New Yorker, supplied by a local grower of Greeley, Colo., who had used his own home-grown seed for a series of years. These types were really only rather imperfectly established size grades, evidently obtained by bin selection from the general field run of his stock.

C.—PARASITIC DISEASE GROUP.

12. The progeny of representative hills from a typical "Fusarium-blight" field of the variety Early Ohio, grown at Greeley, Colo., in 1914, dug in August and stored in a mass lot.

13. Ten hill lots of the variety Early Ohio, grown at Greeley, Colo., in 1914. The physical condition of the soil of the field was poor, and the plants were small and dwarfed.

14. A representative lot from a field of choice stock of the variety Sir Walter Raleigh, grown in 1914 on a field at East Lansing, Mich., which was heavily infected by Fusarium. Every plant in the field, with the exception of about one-quarter of 1 per cent, wilted and died three or four weeks before frost.

15. Sixty-one hill lots of the variety Pearl, grown in Wisconsin in 1914. The hills selected were from vines with more or less rolled foliage and a brown discoloration of the vascular tissue of the stems. Cultures from the discolored stem tissue failed to yield Fusarium.

16. Eighteen hill lots of the variety Pearl, grown from Wisconsin seed at Greeley, Colo., in 1914. Cultural tests at digging time showed unusual infection of the vines with *Fusarium oxysporum*.

17. Six hill lots of the variety Red McClure, grown at Greeley, Colo., in 1914 on vines shown by isolations to be infected with *Fusarium oxysporum*.

18. Forty hill lots of the variety Rural New Yorker, grown on diseased vines at Waupaca, Wis., in 1914. Cultural tests of the vines for Fusarium at digging time yielded a Fusarium and a Colletrotrichum culture in about equal numbers, but these did not appear to be general.

19. Twenty-five hill lots of the variety Rural New Yorker, grown at Greeley, Colo., in 1914 on vines infected with *Fusarium oxysporum*, as shown by isolation tests from the vascular tissue of the stems at digging time.

PRESENTATION OF RESULTS

VASCULAR DISCOLORATION

The number of tubers in each lot of material and the number having discolored vascular bundles, grouped according to the relative depth of penetration below the stolon attachment, are shown in Table I. A column for miscellaneous symptoms is included to provide for a variety of incidental occurrences, such as net necrosis, decay, mechanical injury, and the like; and following this, the number of tubers of each lot with no vascular discoloration is shown.

It has already been stated that, in general, tubers with stem-end vascular tissue of normal appearance were not submitted to culture and that one, two, or three cultures were made from tubers with discolored vessels, the actual number being determined by the depth of the necrosis. No regular procedure was adopted with respect to the tubers belonging to the miscellaneous group. The figures in the column marked "theoretical," under "number of cultures," have been obtained by adding the number of shallow discolorations, twice the number of deep discolorations, three times the number of very deep discolorations, and whatever number the notes show to be correct to provide the cultures made from tubers with miscellaneous symptoms. The actual number of cultures made and reported upon follows in the next column. Under "duplicates" are included the number of cultures made from discolored tubers in excess of the number theoretically required. The number of cultures made from tubers with no discoloration of the stem-end tissue is next recorded, and last of all is given the number of cases in which a culture was theoretically called for but was not reported. In some cases, for one reason or another, these cultures were not made, while in others they were made and discarded before being studied, because of broken tubes, loss of identifying label, and similar accidents. If the number given in the last column is subtracted from the sum of the numbers in the two preceding columns and the difference is added to the theoretical number of cultures, the actual number is obtained.

TABLE I.—*Appearance of vascular tissue and origin of cultures*

Lot No. and designation.	Number of tubers.	Nature of discoloration.					Number of cultures.				
		Shallow.	Deep.	Very deep.	Miscellaneous.	None.	Theoretical.	Actual.	Duplicate.	From tubers not discolored.	Theoretically required but lacking.
OBSCURE DISEASE GROUP											
1 Id	1,731	474	26	4	5	1,222	544	590	44	57	55
2 WPB	387	159	1	1	2	224	164	162	8	13	23
3 Me	636	206	2	0	10	418	217	215	6	14	22
HEALTHY GROUP											
4 CLO	335	233	21	2	9	70	290	373	85	5	7
5 PC	957	563	0	0	2	392	564	572	19	11	22
6 PW	537	80	0	1	5	451	84	89	1	12	8
7 PSW	65	10	0	0	0	55	10	9	0	0	1
8 PMe	133	14	0	0	0	119	14	16	0	2	0
9 RW	360	58	0	0	1	301	58	59	2	5	6
10 RWColl	7	0	0	0	7	0	7	7	0	0	0
11 CRC	664	262	3	0	2	397	269	280	13	9	11
PARASITIC DISEASE GROUP											
12 AEO	213	132	17	0	0	63	166	181	24	2	11
13 EO	69	17	1	1	0	50	22	22	1	1	2
14 M	546	289	6	1	1	249	305	298	10	8	25
15 DPW	391	85	0	0	3	303	88	80	1	7	16
16 DPC	152	88	0	0	0	64	88	106	16	5	3
17 RMe	47	14	0	0	0	33	14	16	1	2	1
18 DRW	222	51	0	0	0	171	51	55	0	5	1
19 DRC	145	61	3	0	0	81	67	73	8	3	5
Total	7,596	2,796	80	10	47	4,663	3,022	3,203	239	161	219

ISOLATION AND IDENTIFICATION OF FUNGI

Isolations were made by transferring a small piece of tissue removed under aseptic conditions from the region of discoloration directly to a test tube containing sterilized nutrient material prepared in the usual way. Melilotus stems, potato cylinders, and steamed rice were used, the number of each diminishing in the order named. Identifications were made direct from the original tube in some cases, while subcultures were resorted to in others. Except in part of the Fusarium cultures, no attempt was made to identify the species. Two hundred and ninety-one out of the 718 cultures of Fusarium secured were identified as *F. discolor* var. *sulphureum* or *F. oxysporum*, but it is not to be supposed that the remaining 499 cultures were all of other species. Indeed, it is probable that *F. oxysporum* and *F. radicicola* predominated among the cultures reported as *Fusarium* spp. The summarized results of the cultural studies are presented in Table II. Two columns of figures appear under each genus reported. In the first column is given the number of instances when the culture was either pure or so nearly so as not to give

visible evidence of the presence of other organisms at the time of identification. In the second column is recorded the number of times the genus in question was found in a tube associated with some other organism. Each tube containing a mixed culture is reported twice, once for each organism. In no case were more than two organisms identified from a single tube. The total number of identifications reported is therefore the sum of all the columns marked "pure" plus the sum of all the columns marked "mixed," while the total number of plantings reported is the sum of all the columns marked pure plus one-half the sum of all the columns marked "mixed."

One very significant thing shown in Table II is the fact that out of 3,203 plantings, all but 161 of which were made from discolored tissue, 1,352 gave no growth. There is good reason to believe that in the great majority of these cases the tubes yielded no growth because the tissue transplanted was sterile, or at least free from filamentous fungi. These results are in entire accord with those obtained by the writer in numerous other cases where cultural tests of discolored vascular tissue of potatoes have been carried out. In some instances the discoloration may be a response to parasitic attack on some other portion of the plant, though the tissues of the tuber are not actually attacked. In such cases it may be regarded as a parasitic phenomenon of a secondary character. From the physiological point of view, however, it matters little whether a lethal dose of toxin diffuses from some point in the stem back of the stolon or from a point within the tuber itself. Likewise, the result is the same whether the tissue is killed by the action of fungi, primary or secondary, or through the operation, directly or indirectly, of malign environment of whatever nature. Conclusions based on field experiments with many factors uncontrolled must not be accepted without reserve, but the writer has secured deep vascular discoloration which he believes to be the direct result of too rapid respiration induced in the soil at high temperatures such as prevail during the summer months in the vicinity of Washington and which are occasionally experienced at more northern and western points. This was the case with stock grown at Arlington Farm during the summer of 1917, in which vascular discoloration was universal and pronounced, extending throughout the tuber in most cases. While certain lots of this material yielded Fusarium or other fungi from a certain portion of the plantings, other lots yielded only an occasional saprophytic growth out of hundreds of plantings. The results were confirmed by repeated trials, which gave uniformly identical results.

There seems, therefore, to be good reason to regard some of the stem-end browning of vascular tissue as physiological, even in the cases in which it extends well into the tubers.

TABLE II.—*Number of isolations from stem-end tissue*

Lot No.	*Alternaria* spp.		*Bacterium* spp.		*Colletotrichum* spp.		*Fusarium discolor sulphurium.*		*Fusarium oxysporum.*		*Fusarium* spp.		*Penicillium* spp.		*Rhizoctonia* spp.		*Verticillium* spp.		Miscellaneous.		No growth.	Total.	
	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.	Pure.	Mixed.		Pure.	Mixed.
OBSCURE DISEASE GROUP																							
1	59	3	26	6	0	1	11	0	8	0	25	6	20	0	3	1	6	5	18	0	493	579	22
2	62	1	17	0	1	0	4	1	1	0	14	2	13	0	0	0	9	1	4	1	33	158	8
3	10	0	2	0	4	6	1	0	0	0	3	1	6	1	0	0	9	0	7	0	155	208	14
HEALTHY GROUP																							
4	25	9	7	4	1	0	4	0	29	0	185	13	2	1	0	0	2	1	5	0	99	359	28
5	181	19	11	4	8	4	12	0	6	0	11	5	47	1	1	0	67	18	12	1	168	546	52
6	12	1	16	0	0	2	1	0	6	1	10	4	0	0	1	0	1	0	1	0	16	84	10
7	1	0	0	6	0	0	0	0	2	2	0	4	0	0	0	0	0	0	0	0	0	3	12
8	3	1	1	0	2	0	0	0	0	0	2	0	0	0	1	0	0	0	0	1	6	15	2
9	7	0	6	7	2	2	1	0	14	1	6	8	0	0	1	0	0	0	3	0	10	50	18
10	0	0	0	2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	4	5	4
11	11	3	24	3	1	0	8	1	8	1	57	2	4	0	2	0	7	1	9	1	143	274	12
PARASITIC DISEASE GROUP																							
12	90	15	3	1	0	0	3	0	8	0	40	14	1	0	0	0	1	0	1	2	18	165	32
13	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	18	22	0
14	0	0	2	3	8	44	1	0	59	43	0	3	0	0	1	0	8	1	18	0	154	251	94
15	13	0	19	5	2	0	2	0	23	0	0	5	0	0	1	0	2	0	0	0	14	75	10
16	43	3	6	1	2	0	1	0	0	0	2	0	4	0	1	0	1	2	2	0	41	103	6
17	2	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	5	1	1	0	5	15	2
18	5	0	12	1	0	0	0	0	26	0	2	1	0	0	0	0	0	1	0	0	11	54	2
19	3	1	4	0	0	0	1	0	0	0	2	1	1	1	0	0	6	1	0	0	54	71	4
Total	550	65	126	45	32	59	51	2	190	48	358	71	100	4	11	1	116	31	81	6	1,352	3,037	332
Total for genus		615		241		91		53		238		429		104		12		147		87	1,352		3,369

Another striking thing brought out in Table II is the frequency with which Alternaria was recovered from the vascular tissue. Almost 20 per cent of the discolored tubers carried this genus, in most instances unmixed with other fungi. This proportion is so high as to suggest that it may possess some significance hitherto unsuspected or at least undiscovered. Similar results have frequently been secured with other material. As high as 50 per cent of some lots of tubers have yielded Alternaria in cultural tests, even from stock presenting an attractive appearance on superficial examination.

FIELD STUDIES

The general manner in which the stock was handled in planting has already been indicated (p. 277–278). In taking notes in the field a full description of each plant was recorded at each reading, including such matters as size, habit, character, color, and orientation of stems and foliage, as well as the general appearance as to vigor. At least three sets of notes, and in the case of some lots more, were made on each plant during the season. Successive sets of notes were taken by different members of the staff, and no reference to the previous notes was made while preparing the new set. In the preparation of the present article the writer has endeavored to translate these descriptions into the expressions "diseased" and "healthy." Every plant has been placed in one group or the other, even though in some cases the assignment had to be more or less arbitrary. Consistency has been maintained, however, and the writer has been able to bring to his aid thorough familiarity with the appearance of the material throughout the season. One of the three principal sets of notes is his own.

Plants whose description at any given note taking indicates probable suspicion in the mind of the observer of the presence of disease have been recorded as diseased, even though at previous or subsequent note takings they may be recorded as healthy. It is certain that many cases of recorded disease at the first note taking represent only delayed germination, but as this may be correlated with reduced vitality or fungous attack on the sprout or tuber, it seems important to record it. Records of recovery as well as of disease have been made and will be considered later, but it is of interest first to inquire into the general relation of vascular discoloration to fungous invasion and the correlation of these within the tuber with disease in the plants produced. For the purpose of this consideration plants once reported as diseased have been counted as diseased whether later reported as diseased or healthy.

RELATION OF VASCULAR DISCOLORATION TO FUNGOUS INVASION AND DISEASE

Table III is designed to show the performance in the field of all the tubers studied, arranged according to the character of the tubers. The tubers are grouped under four headings:

1. Tubers with vascular discoloration yielding a culture.
2. Tubers with vascular discoloration yielding no culture.
3. Tubers without vascular discoloration yielding no culture.
4. Tubers without vascular discoloration yielding a culture.

The tubers under each heading are arranged in two columns, according as they yielded plants which were healthy or diseased. In case a tuber was cut into two or more pieces at least one of which produced a diseased plant, the tuber has been reported in the disease column. As is to be expected, most of the plants in the progeny of the lots carrying obscure tuber-borne diseases are diseased. The results presented in the remaining two groups, however, seem to indicate that vascular discoloration does not necessarily imply fongous invasion; nor is either of these in the tuber a guarantee of disease in the plant, or their absence a guarantee of health.

TABLE III.—*Number of healthy and diseased plants from tubers examined*

OBSCURE DISEASE GROUP

Lot No.	Discoloration; fungus present.		Discoloration; fungus absent.		No discoloration; fungus absent.		No discoloration; fungus present.		Total.
	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	
1	15	147	23	324	86	1,124	0	12	1,731
2	22	90	14	37	42	171	2	9	387
3	8	46	22	142	73	339	2	4	636
Total	45	283	59	503	201	1,634	4	25	.....

HEALTHY GROUP

4	128	65	53	19	55	10	4	1	335
5	263	118	131	53	261	122	6	3	957
6	28	33	12	13	249	190	8	4	537
7	5	4	1	0	22	33	0	0	65
8	8	0	6	0	114	3	2	0	133
9	19	25	6	9	151	146	2	2	360
10	3	0	6	4	0	0	0	0	7
11	82	46	74	65	216	175	5	1	664
Total	536	291	283	163	1,068	679	27	11	.....

TABLE III.—*Number of healthy and diseased plants from tubers examined*—Continued

PARASITIC DISEASE GROUP

Lot No.	Discoloration; fungus present.		Discoloration; fugus absent.		No discoloration; fungus absent.		No discoloration; fungus present.		Total.
	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	
12	113	18	16	2	52	9	2	0	212
13	2	5	4	8	34	15	1	0	69
14	76	62	76	83	133	113	2	1	546
15	20	39	7	22	115	182	0	6	391
16	17	43	10	18	29	31	2	2	152
17	3	6	3	2	12	20	1	0	47
18	17	25	4	5	93	74	1	3	222
19	5	12	23	24	29	50	0	2	145
Total	253	210	143	164	497	494	9	14	.....
Grand total	834	784	485	830	1,766	2,807	40	50	7,596

INFLUENCE OF ENVIRONMENT

Influence of environment upon the development of disease and recovery is a subject of much interest and importance. Table IV brings out some interesting facts regarding the development of disease in Wisconsin and in Colorado in cut and uncut seed. It should be borne in mind that the plants grown in the two States from cut seed are from the same individual, since, as has already been stated, the tubers were halved lengthwise and one half was planted in each place. The seed under 3 ounces was not cut but was divided into two approximately equal portions for planting. For the cut seed the total number of tubers cut and the total number of seed pieces appear in each line. One-half the number of seed pieces is the number planted in each State, except from lot 3. This lot was planted in Colorado only, and it was halved crosswise into stem and apex pieces instead of lengthwise.

The third and fourth columns give the number of diseased plants developing in Wisconsin and Colorado, respectively, and the following column gives the number of cases in which corresponding portions of a given tuber yielded diseased plants in both places. These plants are referred to as pairs. In No. 3 only, the pairs are from stem and apex halves of the same tuber. Of the 197 diseased plants recorded, 106 were from stem-end seed pieces and 91 were from apex or seed ends. As shown in the table, 79 pairs occurred.

For the uncut seed the number of tubers planted in each State and the number developing disease in each State appear.

TABLE IV.—*Distribution of diseased plants*

OBSCURE DISEASE GROUP

Lot No.	Cut seed.					Whole seed.			
	Number of tubers.	Number of seed pieces.	Number of diseased plants in Wisconsin.	Number of diseased plants in Colorado.	Number of diseased pairs.	Number of tubers in Wisconsin.	Number of diseased plants in Wisconsin.	Number of tubers in Colorado.	Number of diseased plants in Colorado.
1	757	1,636	729	698	650	483	432	491	454
2	11	22	8	9	7	194	181	182	116
3	143	286	.....	197	79	.....	.....	493	414
Total	911	1,944	737	904	736	677	613	1,166	984

HEALTHY GROUP

4	184	376	52	45	25	75	12	76	11
5	631	1,552	105	201	42	163	24	163	42
6	482	1,076	177	141	62	27	4	28	3
7	46	114	17	35	12	9	3	10	3
8	10	20	0	0	0	60	1	62	2
9	300	718	104	153	64	30	11	30	5
10	7	14	3	2	1	0	0	0	0
11	84	202	10	73	8	290	82	290	142
Total	1,744	4,072	468	650	214	654	137	658	208

PARASITIC DISEASE GROUP

12	23	46	1	2	0	96	13	93	13
13	5	10	0	1	0	34	13	30	14
14	484	1,136	113	197	52	30	11	32	8
15	125	272	56	74	41	121	75	145	91
16	28	56	19	17	14	63	41	62	31
17	15	30	10	4	3	17	13	15	4
18	96	214	40	37	17	52	25	74	25
19	61	150	31	45	23	45	14	39	26
Total	837	1,914	270	377	150	458	205	490	212
Grand total	3,492	7,930	1,475	1,931	1,100	1,789	955	2,315	1,404

The figures given in Table IV indicate no conspicuous relation between the character of the tuber used for seed and the occurrence of disease, since the number of pairs of diseased plants is only equal to from one-half to one-third the total number of diseased plants in either locality. It is to be noted that in general the Colorado conditions resulted in more disease than did those of Wisconsin, particularly when cut seed was used, and this, too, notwithstanding the fact that the cut tubers were well suberized when planted.

These results seem to indicate that the soil and not the tubers should be considered the most potent source of disease, a fact substantiated for the Greeley section by the more recent studies of Dr. MacMillan. Additional indication of this probability is given in Table V, where the behavior of stem and apex seed pieces is presented and the number of diseased plants per tuber is shown. The obscure disease group, of course, shows a majority of cases in which all the plants from a tuber were diseased, when any of them were; but the combined results from the healthy and the parasitic disease groups show that out of 283 quartered tubers yielding diseased plants, 123 yielded 1 such plant only, 99 yielded 2, 33 yielded 3, while only 28 yielded 4.

TABLE V.—*Number of tubers yielding diseased plants*

Lot No.	From stem pieces.	From apex pieces.	From both stem and apex pieces.	Total number of tubers.	Total number of tubers yielding diseased plants.	Number of tubers yielding 1 diseased plant.	Number of tubers yielding 2 diseased plants.	Number of tubers yielding 3 diseased plants.	Number of tubers yielding 4 diseased plants.
OBSCURE DISEASE GROUP									
1	51	51	49	[a] 57	53	3	5	3	42
2	.....	.....	.....	0	.....	.....	.....	.....	.....
3	106	91	79	[b] 143	118	39	79	.....	.....
HEALTHY GROUP									
4	1	1	0	[c] 4	2	1	1	0	0
5	71	33	27	[d] 141	77	44	20	5	8
6	48	25	23	[c] 56	50	17	18	12	3
7	11	9	9	[c] 11	11	1	6	1	3
8	.....	.....	.....	0	.....	.....	.....	.....	.....
9	46	23	21	59	48	20	14	11	3
10	.....	.....	.....	0	.....	.....	.....	.....	.....
11	16	13	12	[c] 17	17	5	12	0	0
PARASITIC DISEASE GROUP									
12	.....	.....	.....	0	.....	.....	.....	.....	.....
13	.....	.....	.....	0	.....	.....	.....	.....	.....
14	41	26	18	[c] 84	49	24	16	3	6
15	9	7	6	11	10	2	6	0	2
16	.....	.....	.....	0	.....	.....	.....	.....	.....
17	.....	.....	.....	0	.....	.....	.....	.....	.....
18	6	3	3	11	6	2	3	0	1
19	9	10	6	[c] 14	13	7	3	1	2

[a] Two tubers were cut into eight pieces each. All yielded diseased plants. Other tubers were cut into four pieces each.
[b] Tubers were cut into two pieces each.
[c] Tubers were cut into four pieces each.
[d] Four tubers were cut into six pieces each. All produced healthy plants, except one stem and one middle piece from the same side of one tuber. These are both recorded as stem plants. Other tubers were cut into four pieces.

It appears further from the data given on the second and third groups in Table V that a tuber from healthy parentage or from fungous-invaded parentage is more likely to yield a diseased plant from a stem-end seed piece than from the apex. Two hundred and fifty-eight tubers yielded diseased plants from stem ends and 150 yielded diseased plants from apex ends. One hundred and twenty-five of these tubers yielded diseased plants from both stem and apex. The ratios, therefore, of stem, apex, and pairs were approximately 10:6:5. The fact that the proportion of diseased stem plants to diseased apex plants is slightly higher in the healthy group than in the parasitic disease group is not inconsistent with other data presented in this paper.

The facts seem to indicate that the greater liability of stem-end plants to disease results not because the vascular tissue of the seed piece is more often infected by fungi but because it is more often endowed with less physiological resistance.

DISEASE AND RECOVERY

Data dealing with disease and recovery are presented in Table VI. The total number of plants reported at the first note taking as diseased is recorded in the first column. Following this is recorded the number of these plants which subsequently appeared to recover and to remain healthy. The next column gives the number of additional plants reported diseased at the second note taking, followed similarly by the number of those which subsequently recovered. The next column records the number of hitherto healthy plants which appeared to be diseased at the third note taking.

In the lower portion of the table the Rural New Yorker and the Pearl varieties have been summarized in juxtaposition for purposes of convenient comparison. The outstanding feature of this table is the remarkable degree of recovery shown, particularly in Colorado. This is especially noticeable with the Pearl stock in Colorado. It is, possibly, the ability of the Pearl to recuperate in that section which accounts for the popularity of this variety in the Greeley region.

A summary of the data on disease and on recovery for the entire experiment in total and by States is given in Table VII. Table VIII shows percentage data figured from information shown in Tables IV and VII. Attention is directed to the figures in Tables IV and VII in connection with the percentage averages in Table VIII, because percentage figures may be misleading when the numbers from which they are computed are small. A striking example of this is shown in Table VIII, where one plant in Wisconsin was diseased and did not recover, while two were diseased in Colorado and both recovered. This appears in the respective columns on recovery as 0 and 100 per cent. In the larger groups and in the aggregates, however, reduction to percentage gives a clearer presentation of the facts.

TABLE VI.—*Disease and recovery*

OBSCURE DISEASE GROUP

Lot No.	Number of diseased plants.	Wisconsin.					Colorado.					Number not recovered.	
		Number in first note taking.		Number added in second note taking.		Number added in third note taking.	Number in first note taking.		Number added in second note taking.		Number added in third note taking.		
		Diseased.	Recovered.	Diseased.	Recovered.	Diseased.	Diseased.	Recovered.	Diseased.	Recovered.	Diseased.	Wisconsin.	Colorado.
1	2,373	943	67	170	31	48	824	97	263	24	65	1,063	1,031
2	324	15	1	112	15	62	93	73	32	32	0	173	20
3	611	.....	.....	.....	.....	.....	372	41	237	29	2	.....	541
Total	3,238	958	68	282	46	110	1,289	211	532	85	67	1,236	1,592

HEALTHY GROUP

4	120	34	11	19	3	11	53	23	0	0	3	50	33
5	372	29	14	54	16	46	177	113	28	14	38	99	116
6	325	67	18	13	5	101	127	50	13	8	4	158	86
7	58	2	1	18	13	0	32	12	5	3	1	6	23
8	3	0	0	1	0	0	2	2	0	0	0	1	0
9	273	55	26	51	41	9	135	74	22	18	1	48	66
10	5	2	1	0	0	1	2	2	0	0	0	2	0
11	307	3	1	75	62	14	208	182	4	3	3	29	30
Total	1,463	192	72	231	140	182	736	458	72	46	50	393	354

PARASITIC DISEASE GROUP

12	29	14	12	0	0	0	5	3	9	6	1	2	6
13	28	1	1	9	0	3	3	3	1	1	11	12	11
14	329	26	8	87	63	11	184	143	12	4	9	53	58
15	296	25	2	65	3	41	35	22	125	101	5	126	42
16	108	35	1	6	0	19	34	13	8	3	6	59	32
17	31	1	0	22	7	0	2	2	6	3	0	16	3
18	127	20	4	44	35	1	43	24	16	16	3	26	22
19	116	6	0	38	12	1	55	49	2	1	14	33	21
Total	1,064	128	28	271	120	76	361	259	179	135	49	327	195
Healthy Pearl	758	98	33	86	34	147	338	177	46	25	43	264	225
Diseased Pearl	404	60	3	71	3	60	69	35	133	104	11	185	74
Total	1,162	158	36	157	37	207	407	212	179	129	54	449	299
Healthy Rural New Yorker	585	60	28	126	103	24	345	258	26	21	4	79	96
Diseased Rural New Yorker	243	26	4	82	47	2	98	73	18	17	17	59	43
Total	828	86	32	208	150	26	443	331	44	38	21	138	139

TABLE VII.—*Summary of disease and recovery*

OBSCURE DISEASE GROUP

Lot No.	Colorado and Wisconsin.				Wisconsin.			Colorado.		
	Number of tubers.	Number of seed pieces.	Number of diseased plants.	Number of recovered plants.	Number of seed pieces.	Number of diseased plants.	Number of recovered plants.	Number of seed pieces.	Number of diseased plants.	Number of recovered plants.
1	1,731	2,610	2,313	219	1,301	1,161	98	1,309	1,152	121
2	387	398	314	121	205	189	16	193	125	105
3	636	779	611	70	.....	.....	.....	779	611	70
Total	2,754	3,787	3,238	410	1,506	1,350	114	2,281	1,888	296

HEALTHY GROUP

4	335	516	120	37	263	64	14	264	56	23
5	957	1,878	372	157	939	129	30	939	243	127
6	537	1,131	325	81	505	181	23	566	144	58
7	65	133	58	34	66	20	14	67	38	15
8	133	275	3	1	70	1	0	72	2	1
9	360	778	273	159	389	115	67	389	158	92
10	7	14	5	3	7	3	1	7	2	2
11	664	782	307	248	391	92	63	391	215	185
Total	3,058	5,507	1,463	720	2,690	605	212	2,695	858	504

PARASITIC DISEASE GROUP

12	213	235	29	21	119	14	12	116	15	9
13	69	74	28	5	39	13	1	35	15	4
14	536	1,198	329	218	598	124	71	600	205	147
15	391	538	296	128	257	131	5	281	165	123
16	152	181	108	17	91	60	1	90	48	16
17	47	61	31	13	32	23	7	30	8	5
18	222	340	147	79	159	65	39	181	62	40
19	145	234	116	62	120	45	12	114	71	59
Total	1,784	2,862	1,084	543	1,415	475	148	1,447	589	394
Grand total	7,596	12,156	5,785	1,672	5,611	2,430	474	6,423	3,335	1,194
Healthy Pearl	1,692	3,417	758	271	1,640	331	67	1,644	427	207
Diseased Pearl	543	719	404	145	348	191	6	371	213	139
Total	2,235	4,134	1,162	418	1,988	522	73	2,015	640	341
Healthy Rural New Yorker	1,031	1,574	585	410	787	210	131	787	375	279
Diseased Rural New Yorker	367	574	243	141	279	110	51	295	133	90
Total	1,398	2,148	828	551	1,066	320	182	1,082	508	369

TABLE VIII.—*Summary of disease and recovery in percentage*

OBSCURE DISEASE GROUP

Lot No.	Percentage diseased.						Percentage recovered.	
	Cut seed.		Whole seed.		All seed.			
	Wisconsin.	Colorado.	Wisconsin.	Colorado.	Wisconsin.	Colorado.	Wisconsin.	Colorado.
1	89.12	85.33	89.44	92.46	89.24	88.01	8.44	10.52
2	72.73	81.82	93.30	63.74	92.20	64.77	8.47	84.00
3	.....	66.88	.....	83.98	.....	78.43	.....	11.46
Total	88.90	81.08	90.55	84.39	89.64	82.77	8.44	15.69

HEALTHY GROUP

4	27.66	23.94	16.00	14.47	24.33	21.21	21.88	41.07
5	13.53	25.90	14.72	25.77	13.74	25.88	23.26	52.26
6	32.90	26.21	14.81	10.71	32.04	25.44	12.71	40.28
7	29.82	61.40	33.33	30.00	30.30	50.72	70.00	39.47
8	0.00	0.00	1.67	3.23	1.43	2.78	0.00	100.00
9	28.97	42.62	36.67	16.67	29.56	40.62	58.26	58.23
10	42.86	28.57	0.00	0.00	42.86	28.57	33.33	100.00
11	9.90	72.28	28.28	48.97	23.53	54.99	68.48	86.05
Total	22.99	31.93	20.95	31.56	22.49	31.84	35.04	58.74

PARASITIC DISEASE GROUP

12	4.35	8.70	13.54	13.98	11.76	12.93	85.71	60.00
13	0.00	20.00	38.24	46.67	33.33	42.86	7.69	26.67
14	19.89	34.68	36.67	25.00	20.74	34.17	57.26	71.71
15	41.18	54.41	61.98	62.76	50.97	58.72	3.82	74.55
16	67.86	60.71	65.08	50.00	65.93	53.33	1.67	33.33
17	66.67	26.67	76.47	26.67	71.88	26.67	30.43	62.50
18	37.38	34.58	48.08	33.78	40.88	34.25	60.00	64.52
19	41.33	60.00	31.11	66.67	37.50	62.28	26.67	70.42
Total	28.21	39.39	44.76	43.27	33.57	40.70	31.16	66.89
Grand total	38.59	47.01	53.38	60.65	43.31	51.92	19.51	35.82
Healthy Pearl	21.65	27.30	12.36	19.01	20.18	25.97	20.24	47.31
Diseased Pearl	45.73	55.48	63.04	58.94	54.89	57.41	3.14	65.26
Total	24.21	30.29	33.41	36.60	26.26	31.76	13.98	53.28
Healthy Rural New Yorker	25.05	48.82	29.06	45.94	26.68	47.65	62.38	74.40
Diseased Rural New Yorker	39.01	45.05	40.21	45.13	39.43	45.08	46.36	67.67
Total	28.97	47.77	31.65	45.73	30.02	46.95	56.87	72.64

SUMMARY

In the material studied, vascular discoloration of stem-end tissues of Irish potato tubers was not found to be proof of the presence of parasitic fungi. Discolored bundles were often sterile, and fungi were frequently isolated from tissues which appeared normal.

The organisms recovered, in the order of their greatest frequency, were Fusarium 720, Alternaria 615, bacteria 241, Verticillium 147, Penicillium 104, Colletotrichum 91, Rhizoctonia 12, miscellaneous 87.

Out of 3,203 plantings, all but 161 of which were from discolored tissues, 1,352 gave no growth.

The field trials indicate that neither vascular discoloration nor fungus invasion of the tissues of the mother tuber is a guarantee of disease in the resulting plants, nor is their absence a guarantee of health. The soil and not the tuber appeared to have been the more potent source of disease.

Stem-end seed pieces yielded slightly higher percentages of disease than eye-end pieces, evidently because the stem end is endowed with less physiological resistance.

The plants showed a marked capacity for recuperation, which varied with the variety, with the environment, and with the interaction of the two.

CROWNWART OF ALFALFA CAUSED BY UROPHLYCTIS ALFALFAE

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INTRODUCTION

When between the years 1909 and 1914 the so-called crownwart of alfalfa was found scattered through several important alfalfa-growing regions on the Pacific slope of the United States, much interest was aroused. The earliest publication dealing with the disease in South America indicated that it might become of considerable economic importance. The fact that it had attained but limited distribution suggested that prompt study might reveal the possibility of effective measures against further spread as well as means of averting serious loss in the regions already invaded. In 1915 this interest formulated itself in a petition [1] framed by the American Phytopathological Society addressed to the United States Department of Agriculture calling attention to existing conditions and urging work upon this interstate problem. In 1917 it became one of the duties of the senior author to begin work upon this disease. The junior author was associated with the work in 1919, making the field observations, giving especial consideration to the taxonomy and morphology of the causal organism, and preparing all the drawings. This paper is a report of the progress that has been made in the study of this disease.

THE DISEASE

COMMON NAMES

In the United States the disease is commonly know by either of two names, crowngall and crownwart. As will be shown later, the structure of the diseased tissue is that of a true gall, and it was called such in the earlier reports of its occurrence. Later the name crownwart was suggested in order to distinguish the disease from the bacterial crowngall caused by *Pseudomonas tumefaciens*, though it had not then been shown that this disease occurs upon alfalfa in the field. Recently, however, galls have been found by Mr. H. L. Westover on alfalfa in Arizona which appear to be true crowngalls, though complete proof is lacking. In view of the fact that a gall similar in appearance to that caused by *Urophlyctis alfalfae* (Lagerh.) P. Magnus is found upon alfalfa, it is even

[1] Phytopathology, v. 5, no. 2, p. 130–131. 1915.

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more desirable than formerly that the disease caused by Urophlyctis should have a distinctive name. The fact that the name crownwart is well established in usage is much in its favor. It will be seen, however, from facts presented later in this paper that this name is somewhat misleading, inasmuch as the galls are not typical warty growths, nor are they formed from the tissue of the so-called crown of the plant in a manner comparable with that in which crowngalls are formed. A name more truly distinctive is suggested by the French name used by Arnaud (*1*),[1] "La Maladie des tumeurs marbrèes de la Luzerne." An English equivalent, marbled gall of alfalfa, the word marbled referring to the mottled effect produced by the brown spore masses seen when any of these galls are cut, would call attention to the one distinctive character of these galls observable at any time and would be accurately descriptive.

HOST PLANTS

Of the many species of the genus Medicago introduced into the United States, *Medicago sativa* is the only one on which the disease has been found commonly. McKee (*17*) found it also on *M. falcata*. The two species, grown near together at the Plant Introduction Field Station at Chico, Calif., seemed to be about equally infected.

Spegazzini (*32*) records the fungus as occurring on *Medicago denticulata* and species of Adesmia in Argentina. Hauman-Merck (*11*) also records the fungus on *M. denticulata* from the same locality and further states that it does not occur upon alfalfa. In view of the fact that search has not revealed the fungus upon *M. denticulata* in the United States even when the plant is growing abundantly close in association with diseased alfalfa, it seems advisable to hold it an open question whether the fungus found in Argentina upon *M. denticulata* and *Adesmia* spp. is identical with that which causes the disease of alfalfa.[2] Thus, the evidence at hand, while it is inadequate for the formation of final conclusions, appears to indicate that the species of Urophlyctis occurring on *M. sativa* is probably limited to that species and to *M. falcata*.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The only available information regarding the economic importance of the disease consists of expressions of opinion based on a larger or smaller amount of field observation. The trend of the opinion that has developed from this observation is that the disease is, or becomes locally, very destructive to alfalfa plants.

The first report of the disease by von Lagerheim (*14*) from Ecuador gave inception to this trend. He states that diseased plants can easily

[1] Reference is made by number (italic) to "Literature cited," pp. 321–323.

[2] A portion of a collection of *Urophlyctis alfalfae* var. *adesmiae* on *Adesmia bicolor*, sent by Spegazzini to the Office of Pathological Collections, Bureau of Plant Industry, has been examined and been found to contain a Synchitrium rather than a Urophlyctis.

be distinguished in the field, and his illustrations of diseased plants with crowns encrusted with large galls contributed effective support to his statements. However, von Lagerheim states that he did not see the disease in the field himself, though he sought for it in fields near Quito. He received his specimens from the owner of an estate in the Andes, and his description of the effects of the disease in the field was gathered from several observers.

In Europe, Magnus (*20*) reports a destructive outbreak of the disease in Alsace, basing his report on the observations of two farmers. Later, from an adjoining Province of Germany, Grimm and Korff (*10*) report the disease as present in an alfalfa field without causing much apparent harm. In fact, the diseased plants seemed somewhat more vigorous than the others. Nevertheless, they think measures should be taken to eliminate it.

Peglion (*25*) finds the disease in Italy, and raises the question whether or not it may be a factor in producing alfalfa sickness in some fields. He suggests that experimental work should be undertaken to determine the matter. In France Arnaud (*1*) reports the disease as apparently doing considerable damage in a single field in the Department of Seine-et-Oise. In 1916 Salmon (*27*) found a single field infested with wart in England and urged further search for the disease. No reports of serious infestations have followed, though the writers have been told that occasional specimens are found. The disease has been found in Holland (*8*) and Sweden, but no apparent damage has been reported.

A critical reading of these reports of the destructive action of the disease calls attention to the fact that the two most important reports, those of von Lagerheim and of Magnus, are not based on first-hand observation. In all cases damage is noted only in small areas. Therefore we must still hold it an open question whether this disease has been primarily responsible for any serious or widespread injury to alfalfa in either South America or Europe.

In the United States the disease has been found abundant only west of the Sierra Nevada and Cascade Mountains, though it occurs in a few regions east of these mountains. It has not been found east of the Rockies. However, in view of the fact that the disease when not abundant is often completely concealed unless a plant is uprooted, it is possible that its distribution is more widespread than records show.

The first report of the disease by Smith (*31*) gives no clue to its importance. O'Gara (*22*) finds the disease very common and occasionally destructive in fields in the Rogue River Valley in Oregon. Jackson (*12*) later reports the disease from the same region, making no comment regarding its importance. Again O'Gara (*23*) is first to report the disease present in the Salt Lake Valley in Utah, though he has not in this case determined to what extent it causes injury. McCallum (*16*) reported the disease present in Arizona. McKee (*17*), who has had an opportunity

to observe the disease extensively, concludes that crownwart decreases the yield and shortens the life period of plants. He says:

> Alfalfa fields that had the crown wart in abundance in 1914 produced good crops of hay in that year and in 1915. In one field sown in 1910 that has been under observation the past two years, practically every plant has galls. This field has produced apparently normal crops of hay, but more critical observation shows decreased vigor in the plants and a corresponding decrease of yield.

McKee believes the disease much more widespread than is commonly supposed and urges work to determine its importance in alfalfa culture.

Thus it appears that although the disease is scattered through large alfalfa-growing areas in the United States, yet it does not appear at any place to have become regarded as a serious limiting factor in the growth of the crop, except during years of severe attack and even then in small areas.

The writers have not attempted to determine the present limits of spread of the disease in the United States. A limited amount of time has been spent in the spring of three years observing the disease, chiefly in the river valleys where it is known to be most abundant, the Sacramento River Valley in California and the Rogue River Valley in Oregon. The second of these years, 1918, appears to have been distinctly unfavorable for the development of the disease, especially in California. The winter rainfall was below normal, in consequence of which the Sacramento River did not overflow its flood plain where McKee observed the disease to be most abundant. The disease was commonly present on a larger or smaller percentage of plants, but nowhere did observation bring conviction that considerable damage was being done. In the San Joaquin Valley that year only occasional diseased plants could be found, though in some localities there was excellent testimony from farmers of the abundance of the disease in previous years.

In 1919 there was much more winter rain, especially in the Sacramento River Valley, and a greater amount of disease was found. Even then it was only rarely that the disease was sufficiently abundant to appear to be of serious economic importance. Plants could be found whose early buds had become so completely infected that few were left to form the second and later cuttings, but such plants were usually widely scattered among others less severely infected. Rarely indeed does the disease appear to be solely responsible for the killing of entire plants, though it must often weaken them. A significant estimate of the actual damage done can be made only after careful observation has extended over a period of years when the varying intensity of the annual attacks can be studied and the behavior of the diseased plants followed throughout the year

DESCRIPTION OF THE DISEASE (PL. 47)

The disease is more easily described by stating briefly the origin and method of development of the galls. So far as the writers can discover

all galls as they occur naturally in the field result from the infection of buds in early stages of development as they emerge from the crown of the plant. It is well known that there is an almost continuous succession in the development of buds from the so-called alfalfa crown during the entire year. A portion of those buds which will produce the shoots furnishing the first crop in the spring have begun development as early as the preceding autumn. Generally speaking, the first buds to be formed in the seasonal succession have a point of origin deeper in the soil than those which are formed later, so that many of the buds from which the shoots of the third cutting arise develop from positions quite above the surface of the soil. Buds produced below the soil level in cool weather appear to have a meager protection of scaly covering, and it is for the most part such buds that become infected and give rise to galls. Thus, galls are swollen and distorted bud elements, scales, leaves, and stipules.

Unless overwintered galls which are described later are discoverable, the disease is first evidenced in the spring by a slight thickening and rounding of the young buds. During two years this has been observed near Chico, Calif., in the latter part of March or early in April. The diseased buds become more and more rounded as growth progresses and are glistening white in color (Pl. 54, A). Then, as the infected structures begin to push apart, some of them grow much more rapidly than others until the structure as a whole assumes a conspicuously irregular form. In most cases, however, an examination of the gall will show that it is made up of thick, scalelike layers about a central growing axis (Pl. 36, B). Sometimes this axis continues growth in spite of the demands of the mass of developing gall tissue and produces a weak shoot. The earlier and more vigorous buds produce the larger galls. Smaller galls often appear to be developed from smaller buds along the stems below ground that would ordinarily remain dormant. The origin of galls that appear on stems several inches above the surface of the ground in wet weather appears to be due in part to the infection of axillary buds that would never develop in the ordinary course of events and in part to the elongation of the stems and petioles which force infected tissue upward.

Since a large part of the infected buds are developed at a depth of 2 or 3 inches below the surface of the soil, the majority of the galls are so far below ground that they escape observation unless the soil is removed from around the plant. If they are of large size some of them come to the surface, where they take on a green color and in extreme cases form a crust of diseased tissue around the base of the healthy stems.

Another type of gall that is not common results from local infections on young leaves. Such infections give rise to small blister-like galls much like those produced on *Sanicula* spp. by another species of this fungus which will be mentioned later.

The galls reach full development (Pl. 54, B) early in the summer, in early June in northern California. From this time on the majority of them begin to decay if moisture is abundant, or to shrivel and dry with the coming of drouth. However, in almost all fields a few galls more deeply situated become covered with a corky layer and survive the winter.

When plants are subjected to dry conditions in late summer, as they usually are in the Rogue River Valley in Oregon, many of the galls do not decay but remain living throughout the autumn and winter. It does not appear that such galls make appreciable growth during the following year. Nevertheless, gall tissue may accumulate around old plants in considerable mass. The exterior becomes covered with a brown, corky layer that has a much warted appearance. This accumulation of gall tissue has not been found on plants that have grown in well-irrigated fields.

At whatever age or state of development these galls are found, they possess one distinctive character that is discovered when they are cut open. The interior of the galls contains many small, irregularly shaped brown masses of fungus spores which are easily visible (Pl. 56, B). In old dried galls the host tissue has shrunken so much that the spore mass often occupies a large portion of the mass of the gall. Even in decayed galls that have not yet been broken to fragments the spore masses can be recognized by their golden brown color.

CAUSAL ORGANISM

NOMENCLATURE

Some difference of opinion concerning the identity of the parasite causing crownwart of alfalfa has prevailed. Von Lagerheim (*24*) seems first to have regarded it as a new and distinct species, which he cited as *Cladochytrium alfalfae*. Later, however, he (*14*) identified it with *Urophlyctis* (*Physoderma*) *leproidea*, a parasite causing conspicuous malformations on the beet, originally described from Algeria by Trabut (*34*) and assigned by him as well as by Saccardo and Mattirolo (*26*) to a new Ustilaginous genus, Oedomyces. In making this disposition, von Lagerheim opposed the views of both Vuillemin (*35*), who had identified Trabut's beet organism with *Urophlyctis* (*Cladochytrium*) *pulposa* (Wallroth), long known to be parasitic on species of Chenopodium and Atriplex, and of Magnus (*18–20*), who later came to regard the parasites on *Chenopodium* spp., on the beet, and on alfalfa as three distinct species. None of these views appear to be based on evidence altogether conclusive; nor can we adduce such evidence here, because the lack of fresh diseased material of beet and of *Chenopodium* spp. have made it impossible to attempt cross-inoculation experiments.

Provisionally, it appears advisable to follow Magnus in recognizing the alfalfa parasite as a distinct species, not, perhaps, so much on account of some differences in morbid host anatomy as because of the general improbability that two unrelated plants serve as hosts to a parasite which shows in general no omnivorous tendencies. The beet disease has not been reported in the regions where crownwart is prevalent; and *Chenopodium* spp. with every chance for infection have not been observed to be attacked. Reference has been made in another connection to Spegazzini's (*32*) report of crownwart on *Medicago denticulata* and its absence from alfalfa in the same range. This condition could most readily be attributed to the existence of another species producing similar galls.

DEVELOPMENT AND MORPHOLOGY OF THE FUNGUS

The morphology of the crownwart organism has not hitherto received much attention. Magnus (*20*) made some observations regarding enlarged hyphae frequently found in old material and referred to the presence of a hyaline cell attached to the concave side of the resting spores; but in the main his specific details concern the pathological anatomy of the host. In more recent years, Wilson (*37*) published a cytological account of *Urophlyctis alfalfae*, arriving at conclusions considerably at variance with those of Magnus. The utilization of old material by both these writers may largely account for their failure to observe important details of development and morphology, as well as explain interpretations that it appears impossible to reconcile with conditions as found in young material much more favorable for study.

GERMINATION OF THE RESTING SPORES

As has long been recognized, the fungus passes through the prolonged periods of summer drouth by means of the resting spores contained within cavities in the galls of the host. In the course of the rainy season the galls disintegrate completely, thus setting free the spores; and it is not improbable that the exposure incident to this method of liberation may be necessary for germination. However, the conditions that may favor germination remain more or less obscure; for although many attempts were made by the writers with spores from freshly gathered material both old and young, as well as with limited supplies of material that may, in addition, have suffered deterioration in transit, the results obtained have been so meager and dubious that this phase of the life history of the fungus must be reserved for a later paper. In a number of preparations an appearance was noted as of resting spores producing a number of subspherical bodies varying from 1 to 9, by the passage of protoplasm through pores in the spore wall. The vesicles that usually attained half the linear dimensions of the spore in some cases were seen

to produce endogenous motile bodies resembling zoospores that later escaped through a number of openings on the distil side of the vesicular wall. As the Van Tiegham cultures in which this process was noticed were usually several days old, the development of bacteria and various protozoa brought into the observations a considerable measure of untrustworthiness. Indications that similar contaminations may have affected the observations of Wilson (37) on *Urophlyctis alfalfae* and of Bally (2) on *U. rübsaameni* are not entirely wanting. Both of these writers describe the resting spore as functioning directly as zoosporangium.[1]

PENETRATION OF THE HOST

Because of difficulties encountered in efforts to bring about infection under artificial conditions, it has not been possible to observe directly the penetration of the host by the germinating zoospore. However, as an abundance of conditions immediately following the entrance of the parasite were found in stained sections of buds, the course of events during the time of invasion can be followed in incipient stages in the same manner as during advanced stages.

Bodies measuring 3 to 4 μ in diameter were frequently found attached or adhering to the scales or developing axis of the bud. They appear to have made their way under the bud scales very close to the most rapidly growing meristem. Unfortunately, no clear figures showing the immediate development of these bodies were observed—a failure attributable apparently to the fact that by the time the galls became noticeable many weeks had seemingly elapsed since the period during which infection took place abundantly. As a result, the earliest demonstrable stage of invasion was represented by the presence of small turbinate bodies (the "Sammelzellen," "corps centrals," "vésicules collectrices," or "vésicules collectives" of other writers) within the epidermal cells of the outer foliar or scale elements of buds exposed to attack, and attached to and perforating the cuticular wall by an elongated beak (Pl. 49, A, *tu-tg*). More than one body may be present in the same epidermal cell, two or three being not unusual; and occasionally a considerable number of contiguous cells may show such evidence of multiple and concentrated attack. The beak manifestly represents the tube proliferated by the zoospore through which the contents of the latter were conveyed into the host cell after the manner prevailing very generally throughout the Chytridiales.

[1] In an article that has appeared since this paper was prepared, Wilson (38) gives a more detailed account of his findings. So far as his account concerns the germination of the resting spores, it appears to differ very considerably from that more recently published by C. Emlen Scott (30), according to whom each resting spore proliferates from 1 to 15 sporangia, the zoospores escaping through a number of tubes in the hyaline wall. With the latter account the observations recorded above are not at variance.

GROWTH OF THE PARASITE

The fungus cell thus produced is first uninucleated and bears at its apex a short, cylindrical projection. As it becomes older it increases in size, the single nucleus divides, giving rise to a multinucleated condition, and the short apical projection proliferates more or less successively three or four terminal branches which are directed nearly at right angles to the primary axis. These branches subsequently proliferate usually three to five secondary branches directed in the same plane or forward. As a result of this continued ramification, the larger cells may be seen to bear at their apices an apparatus consisting of a short axial stalk branching to form a score of ultimate terminations. There can be little doubt that these processes function as absorbing organs and may thus be regarded as haustoria. In stained sections they are often too badly obscured by host protoplasm to be readily distinguishable; but in preparations of material dissected from fresh, living host plants, they may be studied with ease and certainty.

In the meantime the turbinate cell has increased considerably in size and in number of nuclei, the latter usually ranging from 10 to 20 or even more. As no septa have appeared, the parasite is represented at this stage by a simple coenocyte. With the cessation of growth by enlargement, this condition is altered by the appearance of a number of delicate septa, the ultimate number usually ranging from 3 to 5 but occasionally even reaching 7, each of which delimits a peripheral uninucleated mass of protoplasm. As the septa do not appear altogether simultaneously, the first to be inserted represent convex membranes united to the peripheral wall of the turbinate cell along an elliptical line of juncture, the long axis being parallel with the axis of the turbinate cell. The septa inserted later, when the surface of the turbinate cell has been appropriated in considerable measure, are more likely to be in relation to septa previously laid down as well as to the peripheral wall itself. While the protoplasts first delimited thus tend to approach a double-convex, elliptical lenticular shape, the later ones may be more irregular and have several concave facets (Pl. 49, B).

The further development of each of the peripheral protoplasts thus delimited takes place independently of the other protoplasts similarly derived from the same turbinate cell and follows in the main the course described by Maire and Tison (*21*) for *Urophlyctis hemisphaerica* (Speg.) Syd. (*U. kriegeriana* Magnus) and by Vuillemin (*36*) for *U. leproidea*. Material embedded in paraffin, sectioned, and stained shows the protoplasm very slightly contracted away from the septum along the inner surface, and indications of such contraction are present also in freshly dissected material mounted in water (Pl. 48, B, *tb*). This slightly contracted protoplast now pushes out a protuberance from the outer peripheral wall bounding it (Pl. 48, C, D, *tbx*). In those peripheral segments

occupying a position on the side or toward the base of the turbinate cell, the protuberance will invariably take place at some point along the edge closest to the apical end of the turbinate structure; while in the segments on the apical end the protuberance usually occupies a middle position. By the movement of the nucleus and part of the cytoplasm into the protuberance, the tip of the latter becomes somewhat distended. The constricted position now rapidly elongates, resulting in the formation of an attenuated hypha, uniform in thickness and approximately 0.5 μ in diameter (Pl. 48, A–D). The transfer of protoplasm from the peripheral segment to the distended termination continues for some time, until the former has been completely evacuated (Pl. 48, B, D, *ta*).

The elongation of the hypha involves a translatory movement of the termination in a forward direction, from which, however, it may be deflected by a host cell wall, or even reflected back toward the cuticular wall (Pl. 49, B, *tba*). Ultimately elongation ceases, and the terminal distension develops into a turbinate cell entirely similar to the original product of infection, the single nucleus dividing repeatedly to reproduce the coenocytic condition and the branching haustorial process developing from the apical projection, which becomes observable at an early stage during the period of hyphal elongation.

The proliferation of secondary turbinate cells, which tends to be more abundant from the expanded apical end than from regions more nearly basal, thus involves a certain number of lenticular uninucleated masses of protoplasm, always peripheral in position. The larger remaining portion of the contents of the original turbinate cell is consequently not concerned in this process. It may conveniently be designated as the sporogenous cell and always embraces the contents along the longitudinal axis of the spore and as much peripheral protoplasm as is not involved in the peripheral segments. The contents of the sporogenous cell functions in giving rise to a resting spore in the manner described in the following paragraph.

Sooner or later after the segmentation of the turbinate cell has been initiated, the axial haustorial prolongation buds terminally to produce a small globose swelling, which, when it first becomes noticeable, has no demonstrable irregularities on its surface. Later when the swelling or young resting spore has attained a diameter of perhaps 5 μ (Pl. 48, D, *rs*), there are proliferated along a zone midway between the equatorial region and the distil pole from 9 to 15 slender, unbranched, minute processes. The swelling continues to increase in size until it attains the dimensions of the resting spore (about 25 to 35 by 40 to 50 μ), growth in the earlier stages being due mainly to the transfer of protoplasmic contents from the sporogenous cell through the axial haustorial element but later quite largely by the assimilation of food material from the host. Although the surface of the resting spore is rendered impervious by the deposition of a thick wall during the later stages of enlargement, such

assimilation is made possible by the zone of haustorial processes, each of which has in a manner similar to the apical process become branched to form a ramifying apparatus (Pl. 48, A–D, *ra*, *rb*).

DETAILS OF MORPHOLOGY AND CYTOLOGY

The branched haustorial processes with their unusually definite localization, either as a solitary apparatus at the apical end of the vegetative cell or arranged in a well-defined zone between the equator and the distil pole of the resting spore, constitute perhaps the most striking morphological feature of the parasite. Although the literature regarding these structures, especially with reference to their development and orientation on the resting spore, is unsatisfactory, there seems to be good reason to believe that all the other species usually referred to Urophlyctis, as well as many species commonly assigned to related genera, will show complete similarity to *U. alfalfae* in this respect. Thus DeBary (3) in his account of *Physoderma* (*Protomyces*) *menyanthis* states that—

> Auf demselben (distil) Ende der Blasen findet man sehr häufig ein Büschelchen sehr feiner und kurzer in ein Köpfchen endigender Fäden, welche bald verschwinden und über deren Bau und Zweck ich nichts Näheres angeben kann;

and in the figure referred to the appendages are clearly represented at the apices of the obovoid vesicles ("verkerteiformige Blasen"). Lüdi (*15*), who later studied the same fungus, figured a number of unbranched processes arising independently but in close proximity to each other from the apex of the "Sammelzelle"; and in a few cases he represented a hypha arising also independently from the midst of this cluster. Büsgen (*4*) observed the same structure in *Physoderma* (*Cladochytrium*) *butomi* at the apex of the swellings less rich in contents. Like DeBary this author remained uncertain as to their function but considered it probable that the apparatus consists of budding hyphae together with granular host protoplasm. He reported, too, the presence in this species of—

> irregular cylindrical projections which appear early on the spore, and later are not greatly inferior in length to the diameter of the spore. Stained with iodin, a membrane and hyaline contents with a few granules may be recognized. When the spore matures, these break down.

These structures he designated as haustoria and related their function, in our judgement altogether correctly, to the assimilation of food material. His figures, however, with the exception of figure 19, a, which shows a detached branching rhizoid, lack clearness and lead one to believe that probably groups of newly proliferated young turbinate cells were confused with the rhizoids. On the other hand, the haustoria he shows associated with the resting spores of *Physoderma* (*Cladochytrium*) *flammulae* suggest a good possibility of a zonate arrangement similar to that

found in the alfalfa parasite corresponding, for example, to Plate 48, A–D, *ra*, *rb;* although, to be sure, the attachment of the "Sammelzellen" to the convex haustoria-bearing side would be at variance with any close homology. It appears not unreasonable, however, to suspect that Büsgen was in error in regard to this point and that the resting spore may be attached by its concave side, the concavity, as in *Urophlyctis* spp. generally, very probably being opposite the side bearing the haustoria.

Clinton (*5*) noted the presence of a rhizoid-like process on the side of the "Sammelzellen" toward the young sporangium in *Physoderma* (*Cladochytrium*) *maculare* and figured it both as a terminal apical structure before the development of the resting sporangium has been initiated and as a median whorl after the latter has been formed. Regarding its function he states that—

> The exact nature of these processes is not clearly shown, though they seem to bind the sporangium cell to the Sammelzellen.

In his figure 32 he shows a similar process attached to an element that appears to be a young resting spore, although he makes no reference to this condition in the text.

Schroeter (*28*) observed the apical apparatus on the vegetative cells of *Urophlyctis pulposa*, designating it as—

> ein Krönchen, ein Schopf feiner und kurzer, oft verzweigter Protoplasma Anhängsel.

Vuillemin (*35*), who later studied the same species as well as the beet parasite, appears to have recognized the apparatus as consisting of a "trone" bearing terminally a "houppe" of short ramifying processes—the "panache terminale." To these processes and to the haustoria on the resting spores, as well as to the "appareil nourricier" generally, he (*36*) assigned a structure identical to that of the striated muscle fiber of animals. We have not been able to distinguish anything suggesting striation in any portion of the thallus of *U. alfalfae*. The haustoria here, moreover, appear to have a membrane that seems to persist after the contents have been withdrawn by plasmolysis or have degenerated. The history of the development of the haustoria on the resting spore as given for the beet parasite again is at variance with their development as observed in *U. alfalfae*. For the resting spore, according to Vuillemin, comes about by the swelling of the "sommet du trone du panache" in such a way that—

> Les branches se trouvent dissociées en plusiers buissons et entraînées à divèrses hauteurs sur la boule terminale, tandis que d'autres fragments sont restés à la base.

Whereas in *U. alfalfae* the resting spore is initiated as a bud from the tip of the axial haustorial element, never involving translocation of any haustorial ramification. And as has been pointed out, the haustoria on the resting spores are subsequently developed as new structures in a well-defined zone and are not portions of the apical haustorium distributed

in a miscellaneous manner over the surface of the resting spore by the enlargement of the latter.

The time of proliferation of the resting spore seems to be rather variable. It may follow immediately after the septum delimiting the last peripheral segment has been laid down, before the proliferation of the new order of turbinate cells has begun (Pl. 48, B, *rb*), or more usually somewhat later when one or more of the peripheral segments have proliferated secondary turbinate cells (Pl. 48, C, *rb*). Or, as is not infrequently the case with the unusually large primary turbinate cells, the immediate product of infection, the resting spores may not be formed until three or four successions have intervened and the original lesion has become a well-developed cavity (Pl. 50, *tba*). The protoplasm in the sporogenous cells of such primary turbinate structures as well as the host protoplasm of cells or cavities that have long harbored the fungus frequently take a dense uniform stain with safranin—a result that might readily be attributed to the diffusion of a deep-staining substance. Where this abnormal condition becomes very pronounced, it is not improbable that no resting spore is produced at all, the deep-staining protoplasm finally disintegrating in place. With perhaps this occasional exception, every turbinate cell produces always one resting spore. According to Maire and Tison (*21*), *Urophlyctis hemisphaerica* (Speg.) Syd. produces first a succession of "vésicules collectives," each of these in turn giving rise to several others of the next order, until ultimately each "vésicule collective" produces only a single resting spore. Such separation of vegetative and reproductive stages is not discernible in *U. alfalfae*, the production of resting spores being common to each order of turbinate cells; and, although toward the end of the season, when conditions for growth become poor, the proliferation of turbinate cells may be considerably reduced, as may be inferred from the relatively small number of young conditions in old galls containing an abundance of mature resting spores, it is questionable whether their production is ever entirely stopped so long as the host tissue is alive and growing.

In this connection it may be mentioned that the presence of unfavorable conditions for development is indicated usually by a very pronounced enlargement of the hyphae. When the parasite is growing vigorously the hyphae, by which the youngest turbinate cells are attached, do not ordinarily exceed 0.5 μ in diameter. Later their diameter ordinarily increases to 0.8 to 1 μ, the increase being, as Vuillemin (*35*) has pointed out, in the wall, the lumen remaining the same and, indeed, soon appearing devoid of protoplasmic contents. In old, overwintering galls, however, there may be found usually an abundance of hyphae measuring 3 to 5 μ, the surface of which may be marked with irregularities which give the structure a granular appearance, especially in stained paraffin sections. Within these hyphae the turbinate cells occur as loculi in distensions occupying junctional or terminal positions and are connected

with each other by the persisting, very narrow, central lumina (Pl. 52, B). Magnus (*20*) designated this as encysted mycelium and regarded it as being probably viable; although the degenerated condition of the protoplasm where this is present, and more particularly the very frequent absence of any contents whatsoever, would not argue for a high degree of vitality. However this may be, the appearance of such swollen mycelium suggests a pathological condition of the parasite rather than a normal one.

Beyond a statement by Wilson (*37*), quite impossible of interpretation in the light of the life history here presented, that the—

> content, cytoplasm, and the nuclei of the resting spores in the dormant condition corresponds to that of the plasmodium in the stage immediately preceding spore formation,

there appear no cytological allusions in the literature on the alfalfa parasite. However, certain details regarding the nuclear behavior in *Urophlyctis rübsaameni* have been given by Bally (*2*), and the valuable paper on *U. hemisphaerica* by Maire and Tison (*21*) contains a brief account of nuclear changes in the congeneric parasite on *Carum incrassatum* and *Kundmannia sicula*.

The variability in size of the nucleus pointed out by these authors is well exemplified also in *Urophlyctis alfalfae*, the larger and smaller dimensions being here generally characteristic of certain stages in the development of the organism. Thus, in the young primary turbinate cell, the nucleus, which is subspherical in shape, commonly measures about 2 μ in diameter and is composed largely of refringent, nonstainable material and a single, very conspicuous, deep-staining body (Pl. 49, A, *ta-tg*). Later, the nuclei may increase appreciably in size, even before their migration into the secondary turbinate cells or into the young spore (Pl. 49, B, *ta*). Considerable increase, however, appears to take place quite invariably in the single nucleus of the young secondary turbinate cell, a maximum diameter of 5 to 6 μ being here attained before division occurs (Pl. 50, *tb-bx*). Division is initiated by the deep-staining body becoming elongated and being drawn out into a spindle-shaped figure, which may be either straight or distinctly cresentic, depending on the curvature of the portion of the nuclear membrane to which it is laterally applied (Pl. 50, *tbd*). This spindle-shaped structure appears to divide in the middle, yielding two bodies similar to the original, which assume positions separated from each other. A membrane is now formed between the two granules, dividing the nonstainable material about equally; and when the two hemispherical division products have rounded up, the structure of the parent nucleus is reestablished, although pairs of sister nuclei can usually be distinguished for some time by their nucleoles facing each other—a figure that is by no means uncommon (Pl. 50, *tab*).

We have never been able to make out in the nucleus at any stage in the development of turbinate cells anything that would need to be inter-

preted as a chromatin network. Occasionally in nearly evacuated sporogenous cells, where the attenuated condition of the cytoplasm permits of more accurate study, strands were observed close to the periphery of the refringent nonstainable portion; however, from their general appearance and staining reaction, it is much more probable that these represent overlying strands of cytoplasm. The chromatin material here seems to be very largely if not completely concentrated in the conspicuous, densely staining body, which may thus be regarded as a karyosome or chromatin-nucleole. This mode of division presumably constitutes a type of amitosis; and, indeed, with a nucleus of the structure described, mitosis of the regular type is manifestly out of question. And yet the enlongated spindle shape assumed by the nucleole suggests that perhaps division here may involve some mechanism resembling in a rudimentary way the apparatus associated with mitosis. The whole process bears considerable resemblance to that described by Kusano (*13*) as occurring in the zoosporangia of *Olpidium viciae*.

By repeated divisions the nuclei in the turbinate cells reach a number of 10 to 20 before the latter has attained its final dimensions; and this increase in number seems to involve usually a decrease in size, which may sometimes be quite insignificant, or again quite considerable, but is nearly always perceptible. Nutrition seems to have some influence on the size of the nuclei at this stage, the turbinate cells found in recently invaded tissues rich in protoplasm generally remaining relatively large throughout, while those farther toward the origin of the cavity appear to suffer the greatest reduction.

The cytoplasm of the growing turbinate cells stains moderately deeply and seems to have a uniform, finely granular or reticulate structure. During the earlier stages of growth, a relatively large vacuole may usually be distinguished near the proximal end. Perhaps this is later associated with the insertion of a septum near the base of the cell that is probably not always concerned in delimiting a uninucleated protoplast but appears to serve more frequently in shutting off the protoplasm from the evacuated hypha. Although the number of vacuoles of a size readily to be observed may be increased during the later stages of growth to several, the difference between the basal and distil ends never becomes considerable, the structure of the cytoplasm at the time of the insertion of the peripheral septa being generally rather uniformly granular or finely reticulate. The progressive evacuation of contents of both the peripheral segments and the sporogenous cell brings about an attenuation of the cytoplasm which, especially in the sporogenous cell, is associated with the appearance of large vacuoles that ultimately, with the exception of a few strands of cytoplasm, coalesce to fill the entire cell.

As the isthmuses between the peripheral segments and the anlagen of the young turbinate cells, as well as that between sporogenous cell and resting spore, are considerably narrower than the nuclei, the latter

undergo some distortion in their passage through these communications. The achromatin passes into the lumen of the connecting element as a beaked extension followed by the chromatin-nucleole, which, too, is drawn out in a conspicuous manner (Pl. 49, C). The normal nuclear structure is recovered when the material has reached, for example, the flaring portion of the isthmus at the proximal end of the resting spore. The result of the total protoplasmic movement is that in *Urophlyctis alfalfae* the penultimate cells are either evacuated or in the process of evacuation and that all elements more basal in position, hyphae as well as peripheral segments and sporogenous cells, are always quite empty of living material.

Within the young, growing resting spore, the nuclei increase somewhat in size; but much more marked is the immediate increase in size of the chromatin-nucleoles, which at this stage measure 2 μ in diameter, or approximately half the linear dimensions of the nucleus. It is not improbable that some nuclear divisions may take place. In living material the resting spores show a beautifully vacuolate structure, the vacuoles being numerous and relatively large (Pl. 48, A–D, *ra*, *rb*). This structure is apparently poorly preserved in the processes of killing, embedding, and staining. Microtome sections stained with Flemming's triple combination show the cytoplasm as having a dense reticulate structure readily distinguishable, however, even in the earliest stages from the cytoplasm of the turbinate cells by its greater affinity for gentian violet.

Later, during the maturation period, the cytoplasm of the resting spores appears more loosely reticulate, and the nuclei assume still greater dimensions, finally measuring 6 to 8 μ in diameter (Pl. 49, D–F). This increase in size is associated with the appearance of very minute granules of chromatin more or less irregularly disposed near the periphery of the achromatin mass and easily distinguished from the surrounding cytoplasm by a marked difference in staining properties. In many cases the arrangement in a definite reticulum is particularly pronounced (Pl. 49, F). Maire and Tison (*21*) report that in the resting spore of *Urophlyctis hemisphaerica* certain nuclei become enlarged, their nucleoles becoming vacuolated and giving rise to large masses of a substance staining red with safranin which accumulate in the center of the spore. Something similar seems to occur in the maturing resting spores of *U alfalfae*. Plate 49, F, represents an early stage in the process, the three nuclei shown in the center having become conspicuously enlarged, the achromatin having partly lost its refringency, and the nuclear contours having become less distinct. Later, as in Plate 49, F, the chromatin masses are no longer distinguishable but appear to have been transformed or replaced by vacuolate cytoplasm somewhat more attenuated than at the periphery and inclosing in its meshes the numerous granules of red-staining material that have presumably been derived

from the chromatin. Plate 49, D, shows a condition that frequently appears in spores that probably have been poorly nourished. The degeneration of the central nuclei leads to the origin of a large vacuole that ultimately develops into a cavity near the periphery of which a variable number of red-staining granules are always to be found.

Maturation involves, too, a conspicuous transformation and thickening of the wall of the resting spore. Even while growth is still proceeding, the spore wall becomes increasingly thick; and during the later stages of enlargement, although still capable of further distension, in all probability it no longer permits of an easy passage of food materials. After final size is attained, thickening proceeds rapidly. The mature spore wall is a structure about 1.5 μ in thickness, of a yellow, vitreous appearance, inelastic and brittle; when the wall is fractured by pressure applied in manipulation, fragments may break out like pieces of shell from a nut, often leaving the contents quite intact.

When the spore has attained maturity, the haustorial processes disappear, whether by retraction, degeneration, abscission, or accidental fracture could not be definitely determined. However this may be, a circle of pits or scars, corresponding in number and position to the haustoria (Pl. 48, F, G), is always left, because the thickening of the spore wall never involves the places of attachment of the haustoria. In examinations of herbarium material, in which turbinate cells and hyphae are only too frequently quite unrecognizable, these pits serve as a morphological feature of no mean taxonomic value.

GENERAL TAXONOMIC CONSIDERATIONS

The taxonomic relations of the plants included under the genera Urophlyctis, Physoderma, and Cladochytrium remain in need of study. Schroeter (*29*) saw in the association of the "Oosporangium" of *U. pulposa* with the "leere Blase" a sexual apparatus consisting of two conjugating "Fruchtkörper," one of which has yielded its contents to the other. On the basis of this interpretation he erected the genus Urophlyctis, including it with Diplophysa and Polyphagus in the Oochytriaceae, which family he distinguished from all the other families in the Chytridineae not excluding the Cladochtriaceae, under which were brought Physoderma and Cladochytrium by the presence of sexuality in the origin of the resting spores. Fischer (*9*), on the other hand, denied the existence of sexuality in Schroeter's genus and placed it with Physoderma as a subgenus under Cladochytrium. Schroeter's views received support from Magnus, who described a number of forms—*U. kriegeriana* (*18*), *U. leproidea* (*18*), *U. rübsaameni* (*19*), and *U. alfalfae* (*20*)—as congeneric with *U. pulposa* and exhibiting the same type of oogamy. The later investigations on *U. leproidea* by Vuillemin (*35*), on *U. rübsaameni* by Bally (*2*), and on *U. hemisphaerica* by Maire and Tison (*21*) have

not confirmed Magnus' assumption of sexuality in these forms; and from the present account it is obvious that in the formation of the resting spores of *U. alfalfae* there is no indication of any process of conjugation.

In order to determine more nearly in what measure the development and morphology of the alfalfa parasite might be common to related forms, the writers examined herbarium material of various species of Urophlyctis, Physoderma, and Cladochytrium. Fresh living material of a species other than *U. alfalfae* was obtained only from *U. pluriannulatus* (B. and C.) Farlow (7), occurring in the Pacific States on *Sanicula menziesii*, on which host it was collected in excellent condition near Philomath, Oreg., on April 7 and May 16, 1919. As its range extends over the region in which crownwart is known, suspicion has arisen now and then that the two parasites might be identical. This suspicion may now be definitely dismissed.

Urophlyctis pluriannulatus may very easily be dissected from the cavities in the wartlike protuberances on the stems and leaves of diseased plants of *Sanicula menziesii* (Pl. 53). Mounts of thalli consisting of hundreds of turbinate cells and resting spores in a good state of preservation were obtained in this way. Plate 52, A, C, shows two small portions of such a thallus. The general method of development corresponds exactly to that described for *U. alfalfae*, yet morphological differences sufficient to separate the two as distinct species are readily recognizable. Greater dimensions are characteristic of *U. pluriannulatus*, both of turbinate cells (which measure approximately 22 μ in length and 18 μ in major diameter, against 19 μ length and 15 μ major diameter for *U. alfalfae*), and of resting spores, the equatorial diameter here ranging from 45 to 60 μ, as contrasted with 40 to 50 μ for *U. alfalfae*. The turbinate cells of *U. alfalfae* produce usually a maximum of four to five secondary turbinate cells, a greater number being occasionally produced, however, by the very large primary turbinate structures; whereas in *U. pluriannulatus*, turbinate cells not infrequently produce seven or eight turbinate cells of the next order, five or six being the rule. An interesting but rather inconspicuous difference in the structure of the rhizoids on the resting spores may be noted. Since the primary branches are inserted at nearly right angles in *U. alfalfae* while the corresponding angles tend to be much smaller in *U. pluriannulatus*, there is brought about a difference that might crudely be compared, for example, to the difference in habit between a palm and an elm. In *U. pluriannulatus*, too, the haustoria are inserted slightly nearer the equator than in the alfalfa parasite. But the most unmistakable specific difference is to be found in the number of haustoria on each resting spore, which in *U. alfalfae* varies from 9 to 15 and in *U. pluriannulatus* ranges from 14 to 24. (Compare Pl. 48, E, with Pl. 52, D.)

In this connection it may be mentioned that resting spores from herbarium material of all the other species of Urophlyctis examined, after being

boiled with caustic potash and cleared with chloral hydrate, reveal a ring of pits altogether similar to those observed on spores of *U. alfalfae* and *U. pluriannulatus*. That this implies the presence of haustoria in the following species can hardly be doubted:

Urophlyctis bohemica Bubak on *Trifolium montanum*, Rabenhorst-Pazsche, Fungi Europaei et extraeuropaei, No. 4378.

Urophlyctis kriegeriana Mag. on *Carum carvi*, Jaap. Fungi sel. exs. No. 126.

Urophlyctis kriegeriana Mag. on *Pimpinella nigra*, Bubak, F. Fungi Bohemici June 9, 1901.

Urophlyctis magnusiana Neger on *Odontites rubra*, Vestergreen, Mic. rar. sel. No. 1614.

Urophlyctis major Schroeter on *Rumex britannica*, Davis, J. J., Wisconsin fungi. Aug. 27, 1913.

Urophlyctis pulposa (Wallr.) Schroeter on *Chenopodium glaucum*, Sydow Myc. ger. No. 1086.

Urophlyctis rübsaameni Magnus on *Rumex scutatus*, Jaap, O Fungi sel. exs. No. 402.

Seventeen species of Physoderma and Cladochytrium were also examined by the same method, and of these at least 2 species—namely, *Physoderma menthae* Schroeter on *Mentha aquatica*, Vestergreen, Mic. rar. sel. No. 1609, and *P. zeae-maydis* on *Zea mays*, material furnished by W. H. Tisdale—revealed a zone of pits, although no direct evidence could be obtained that these had served as places of attachment for haustoria. It is interesting to note that a certain range in number of pits was found to be characteristic of species and that even numbers seemed to predominate. Thus *Urophlyctis rübsaameni* showed either 6 or 8. Pronounced and constant disparity in number of pits may, indeed, be interpreted as indicating rather clearly that forms assigned to the same species because of close relationship of their hosts may belong to quite different species. It appears hardly admissible, for example, to designate the parasite on *Pimpinella nigra* with 10 to 14 pits as *U. kriegeriana*, when this species of *Carum carvi* shows only from 6 to 10; and the identity of *U. kriegeriana* and *U. pluriannulatus*, suggested by Farlow (7) as a fair possibility, would seem to be equally improbable.

In a number of species as, for example, *Physoderma maculare* (5), *P. butmio* (4), and *P. zeae-maydis* (33), the germination of the resting sporangium involves the lifting off of a circumscribed portion of the spore wall by the expanding endosporangium. Although this "lid" is usually not apparent in the spore wall, its presence on the resting spores of *P. comari*, *P. eleochardis*, *P. gerhardti*, *P. iridis*, *P. menthae*, *P. schroeteri*, *P. vagans*, and *P. graminis* could be determined from an examination of herbarium material with moderate certainty. It remains a question whether the resting spores of those species in which nothing resembling a lid could be made out, including for example, *P. agrostidis*,

P. calami, *P. hipuridis*, *P. spargani*, and *P. speciosum*, germinate, perhaps, in a manner similar to *P. menyanthis*, in which, according to Clinton (5), the outer wall is ruptured by the elongating protoplast, dehiscence of the zoospores taking place at the tip of the protrusion. The absence of any indication of lids from the spores of all species of Urophlyctis examined may be of taxonomic significance, although this can not be determined until more reliable results have been obtained in the germination of the spores. It would be interesting, too, to determine from living material the positional relation between the zone of haustoria and the lid in those species where both appear to be present, as seems to be the case, for example, in *P. menthae* and *P. zeae-maydis*.

The more striking recorded departures of a number of species of Physoderma from the general thallus structure of the two species of Urophlyctis investigated by us remain in need of explanation. One of the departures is found in the septation of turbinate cells and in the fate of the different segments. As has been pointed out, in *Urophlyctis alfalfae* and *U. pluriannulatus* the production of secondary turbinate cells always starts with the delimitation of peripheral segments that involve portions of the parent cell wall, most frequently subapical or lateral and occasionally subbasal. The distinction between a smaller basal cell and a larger distil cell, made by Büsgen for *Physoderma butomi* (3) and by Clinton (5) for *P. maculare*, is thus without significance here; while their accounts of the origin of the resting spore from the proximal cell are directly at variance with developments in *U. alfalfae* and *U. pluriannulatus*, in which the resting spore is invariably developed from the large multinucleate residue not involved in peripheral segments. Lüdi (15) figured the "Sammelzellen" of *P. menyanthis* with 1 or 2 transverse septa and represented the resting spore as being attached to the distil segment thus delimited by a filament of considerable length. According to this writer's account, the resting spore here is not always terminal, but by itself proliferating a "Sammelzelle" it often appears as an intercalary structure associated with two "Sammelzellen." Tisdale's (33) account of *P. zeae-maydis* presents even more points of difference, showing structures consisting of two to four lobulate segments set off by transverse septa, these segments, with the exception of one, capable of forming a resting spore either directly or at the end of a fiber. In this form, organization and development would appear to be of a rather miscellaneous type, contrasting sharply with the definite sequence of growth found in the two plants figured in this paper.

Reference has been made elsewhere to Büsgen's figures of *Physoderma* (*Cladochytrium*) *flammulae*, in which the resting spore is represented as being attached to the "Sammelzelle" by the side bearing the haustoria. Another detail worthy of note in the same figure of Büsgen's is the length of the hypha connecting "Sammelzelle" and resting spore, approximating as it does half the length of the resting spore. In Cornu's

(6) figures of *P. maculare* (*Melanotaenium alismatis*), the hypha connecting "corps central" and spore is even longer, exceeding here the length of the "corps central"; and, as has been indicated above, an entirely comparable figure is given by Lüdi to illustrate conditions in *P. menyanthis*. If these writers have not mistaken turbinate cells (or their homologues) for resting spores and have not erred in relating the latter to the wrong turbinate cells, it would appear that conspicuous variability in length is characteristic of the connecting isthmus which in *Urophlyctis alfalfae* and *U. pluriannulatus* is extremely short.

Magnus emphasized the difference in anatomical effects produced by species he referred to the genus Urophlyctis and by those he assigned to Physoderma. The former cause hypertrophy and thickening of host cell wall, while the latter leave the host tissue in an approximately normal condition. Perhaps a distinction on such grounds would make the classification of parasitic forms contingent in too large a measure on reactions of the host plant to be admissible in a taxonomic sense. It seems not improbable that further study of the plants now referred to Urophlyctis, Physoderma, Cladochytrium, and perhaps a few other related genera will reveal possibilities in generic regrouping based on the more significant similarities and differences in morphology and development.

PATHOLOGICAL MORPHOLOGY

It has already been stated that the fungus attacks primarily leaf scales and leaves at a very early stage of development in the growing bud. Only rarely has it been found to have penetrated to the axis in the dividing undifferentiated tissue of the bud. The stimulative effect of the fungus is limited strictly to the structure which has been invaded, while other structures in the vicinity of the main axis and the axis itself show retardation and often cessation of development.

The first morphological change in the host consequent upon invasion consists in a slight enlargement of the first cell entered so that it comes to project both outwardly and inwardly against the underlying cells. These underlying cells may also show a slight enlargement before they are actually entered by the advancing fungus. The nuclei of the affected cells enlarge notably, and the large deep-staining nucleoles persist for a long time in the fungus cavities, their number serving as an index to the number of host cells that have been destroyed.

The fungus evidently gains access to new cells by the solution of thin cell walls in advance of the growing turbinate cells. In early development when a number of these fungus cells are advancing close together in the same direction, the walls of the host cells are found dissolved before the fungus comes in contact with them (Pl. 55), thus precluding the possibility of mechanical pressure as a factor in effecting the advance. In later stages, however, when turbinate cells are fewer and more scattered,

the host wall does not always yield until the advancing cell is in contact with it, suggesting that mechanical pressure may here be a factor.

The enlargement of cells under the stimulus of the fungus is the smaller factor in the production of galls. As soon as the fungus has begun its advance into the tissue, cell division is stimulated in the vicinity, and even at a considerable distance if the fungus is making rapid growth. The first notable divisions take place in the cells just beneath the epidermis in the region of the point of invasion (Pl. 55). Walls are inserted tangentially to the outer surface of the structure, and the increase in tissue at this point surrounds and may even bury deeply the base of the fungus cavity so that it no longer leads to the exterior of the gall. The thin-walled parenchyma in which the fungus forms its cavities may show little morphological change near the invader in the early stages of its progress, especially if these cells have matured and are not readily capable of division. However, the older part of the surrounding wall of the fungus cavity is soon greatly thickened with a layer which is very brittle when cut and which is therefore poorly preserved in stained preparations. The peculiar structure and markings sometimes found in these walls has been noted by Magnus (*20*), though his assumption that the window-like openings between fungus cavities are due to the local absorption of these walls seems less probable than that they are the partly filled openings through which the fungus advanced at an earlier stage. As soon as this thickening is well under way, the host cells adjoining the cavity begin to divide with walls tending to be oriented tangentially to the wall of the cavity. Such divisions proceed further in the vicinity of vascular bundles than elsewhere, giving rise to a considerable mass of cells in parallel rows, almost cubical in shape, with walls a little thicker than those of the normal parenchyma (Pl. 56, A). But these processes are rarely rapid enough to surround the newer portions of the cavity where the fungus is slowly breaking into cells and extending its ramifying maze. Perhaps the larger bulk of the cells that make up the gall are developed from the vascular bundles where division, especially in later stages in development, becomes very active. Sometimes a bundle becomes much broadened, and from the active cambial region a large mass of parenchyma on one side and a few leaf tracheids on the other are set off. Tissue from this source is likely to be richer in protoplasmic contents than that from the other sources mentioned and is more extensively penetrated by the advancing fungus. Thus, it may be said that the response of the cells to the stimulation of the fungus is in proportion to their capability for meristematic activity and to their nearness to the source of stimulation. Cells near the exterior of the gall divide with walls tangential to the surface of the gall; those in close proximity to the older portions of the fungus cavity divide with walls tangential to the wall of the cavity; while vascular bundles function in division

like stem bundles in giving rise to secondary thickening, producing irregular masses of leaf elements. Thus, the normal limitation in the direction of cell division and growth which produces thin, laminated structures is removed, and thick, fleshy amorphous masses of tissue inclosing ramifying cavities filled with the fungus in all stages of development are produced. On irrigated land these structures are not usually well protected by epidermis or cortex and readily dry out or decay, but in dry regions many become covered with a corky layer that protects them from destruction.

In partial contrast to the galls upon alfalfa is the gall upon *Sanicula menziesii* (Pl. 53) caused by *Urophlyctis pluriannulatus* previously mentioned, a contrast indicated by Magnus (*19*) in his classification of Urophlyctis galls into two types, those upon underground parts of plants and those upon aerial parts. Although the earliest stages in the formation of these galls have not been traced, evidence from more mature stages indicates that the general development is similar to that of galls formed on alfalfa and in fact is exactly like that of the blister-like galls sometimes found on alfalfa leaves. In the attack of the fungus on Sanicula, infection of the leaf, petiole, and stem structures takes place at a later stage of host development than is common on alfalfa, and the response of the host tissue to the stimulus of the fungus is not nearly so great, extending only to a distance of a few cells. Apparently a small number of cells are rapidly invaded soon after the fungus enters the host. Thickening of the host cell walls around the cavity formed, especially its basal portion, soon occurs; and thereafter it appears that a part at least of the enlargement of the fungus cavity is accomplished by the pressure of the growing fungus mass against the surrounding cells, which become flattened and distorted. Thus, each infection produces one partly chambered cavity in the parenchymatous tissue which has become hypertrophied to form a small blister-like gall.

INOCULATION EXPERIMENTS

In order to avert any possible danger of spread of the disease from experimental plots, inoculation experiments were limited to a few potted plants in a greenhouse at Washington and to plants in the greenhouse and on the trial grounds of the United States Plant Introduction Garden at Chico, Calif. At the latter place, perhaps because of the limited time during which work was done there, no success was attained in producing infection. Since one of these failures may be significant, it will be mentioned. On April 15, 1918, nine days after wart was first found developing on plants in the field, an inoculum was prepared by shaking soil and the fragments of decomposed warts from the crowns of a large number of plants which had been badly diseased the previous year and adding a small amount of crushed warts which had been found not yet decayed.

A square yard of vigorously growing alfalfa plants in the corner of a 2-year-old plot was selected for inoculation. These plants were already producing shoots 1 foot or more in height. The soil and débris were carefully scraped away from around the crowns of these plants, exposing a large number of developing buds and shoots. The inoculum was carefully packed around these crowns, the growing tops of which were finally sprinkled and dusted with crushed galls. Sphagnum was packed over and around the plants to a depth of 2 or 3 inches, water was sprayed over the plot, and the sphagnum and soil beneath were kept thoroughly wet for 10 days. On June 1 the material was removed from around the plants, but no trace of any infection was discovered. Whether the rapid growth which the plants were already making at the time when inoculation was made prevented infection or whether some other circumstance was responsible for the failure can not be told until further work is done. From observations which were made in the field, it appears probable that most of the warts which developed that spring resulted from infections which had taken place previous to the date at which the inoculation was made. Thus it is possible that at the late date at which the experiment was begun the spores of the fungus had in large part ceased to germinate, or the plant itself might have passed its period of greatest susceptibility.

Inoculations of plants in the greenhouse at Washington gave two instances of successful infection. In one case a pot of seedling plants about 6 inches tall were inoculated by replacing the dirt around the crowns with crushed diseased tissue and débris from plants recently received from California. Inoculation was made October 1, and on January 3 three plants with very young infections were found.

Attempts to obtain infected plants by sowing seed in soil to which crushed warts had been added usually resulted in the destruction of the young plants by Rhizoctonia and possibly other fungi introduced with the inoculum. In one case, however, among nine plants from seed mixed with Urophlyctis spores and sown in April there were found in the following January three infected plants, two of which were dwarfed and much injured by the disease. If it were possible to obtain a large percentage of plants in the field as badly infected as those in this experiment, this disease would be capable of much harm. As a matter of fact, however, only a relatively small percentage of young plants have been found infected in the field even under what would appear to be the most favorable conditions.

When germination of spores can be obtained with some degree of certainty or when field experiments under suitably controlled conditions can be freely undertaken, opportunity will be open for further infection studies that should add to our meager knowledge of the conditions necessary for infection in the field.

CLIMATE IN RELATION TO THE DISEASE

The fact that the disease has apparently remained so long limited in its distribution to certain regions in the western portion of the country without invading the larger alfalfa-growing areas in the central portion of the country raises the question whether this limitation is due to certain climatic conditions which favor the development of the fungus in these localities or to some factors which have prevented the spread of the causal organism. That the spread of the organism has been inhibited by lack of facilities for distribution is hard to imagine. Even if it should be found that the spores are incapable of withstanding the drying incident to being transported with seed or hay, still a considerable number of plants have been and still are transported by individuals for trial or experimental purposes, and it is hard to believe that no warted plants have been sent at some time into the central and eastern States. On the other hand, it is not easy to discover any common factors of climate in the regions where the disease now occurs which do not exist in the larger eastern regions. For the most part, the disease exists in valleys where the winter is very mild and where there is at least a slight growth of the plant during every month of the year. Such conditions would seem to furnish a long period favorable for infection. However, the disease also occurs in the Salt Lake Valley in Utah and in certain high mountain valleys where the winter is severe. The mere fact of severe winter does not seem to be the sole limiting factor. Thus, it is not possible to answer with an opinion based upon suitable evidence the most important question from an economic point of view that is being asked regarding the disease. Of course it might be determined decisively whether the disease can develop in the central and eastern portions of the country by bringing diseased plants into these regions and observing their behavior. Fear that such experiments might result in a destructive spread of the disease has prevented the initiation of such experiments thus far.

CONTROL MEASURES

Thus far no experimental work bearing directly upon control measures has been undertaken. The direction which such experimental work should take appears to be clearly indicated by the observation of the field conditions under which the disease now becomes most abundant. The one condition which more than any other appears to favor the development of the disease is an excess of moisture in the soil in the early spring when it appears that infection must take place if at all. Any measure which will avert this excess, as by drainage or a diminished supply of irrigation water, should bring about a reduction in the amount of disease.

Under some conditions deep cultivation may reduce the disease. In the spring of 1918 some fields which had received a thorough and deep

cultivation in February were observed to have less of the disease than neighboring fields which had not been so treated. There was ample evidence that the disease had been severe in these fields in the previous season. However, in the following spring the difference between cultivated and uncultivated fields had disappeared.

There is a limited amount of field evidence that the amount of disease is increased when alfalfa is planted directly after alfalfa. Fortunately, such succession is rarely practiced. Thus, on the whole, it can be said that when conditions are made most favorable for the development of the alfalfa plant the disease is diminished, perhaps not so much because the plant is better able to withstand its attacks as because abundant infection is dependent upon conditions which are not of themselves most favorable for plant development.

Search has been made in vain for any evidence of conspicuous cases of apparent resistance to the disease. In one instance in 1919 a plot of alfalfa was found conspicuously freer from the disease than the adjoining plots which appeared to be under exactly the same conditions. It was found that the seed used in this plot was from a different source than that used in the other plots, and in fact the type of plant was different. An effort to obtain seed from this field for experimental work was frustrated by the ravages of grasshoppers. During the following year observation failed to discover any material difference in the amount of disease in this field as compared with its neighbors, and therefore efforts to obtain seed from it were abandoned.

It hardly need be said that until it is known for a certainty whether the disease can be troublesome in the eastern alfalfa-growing regions, care should be taken to prevent its introduction. At least living plants from fields where the disease is known to occur should not be transported to other localities.

SUMMARY

The disease of alfalfa caused by the fungus *Urophlyctis alfalfae*, commonly known as crownwart, has been found to have its origin in the infection of very young buds, the foliar elements of which develop into abnormalities not involving the mature structures of root or stem.

Infection appears to take place only early in the spring, becoming easily discoverable in the latter part of March or in early April in northern California.

In irrigated regions, or in regions where there is abundant moisture during the entire season, most of the galls reach full development early in the summer and thereafter decay rapidly, only a few surviving until the next spring.

The thallus of the fungus consists of two types of structures, turbinate cells and resting spores. In the first turbinate cell that is the immediate development of the infecting fungus are inserted a number of septa

which delimit uninucleated peripheral segments from a polynucleated central sporogenous mass. A hyphal structure of limited growth develops from each of these segments and carries the nucleus in its expanded termination, the latter constituting a young turbinate cell of the next succession. At its mature stage the turbinate cell bears a branched apical haustorium, the short axial element of which proliferates at its tip a globose terminal expansion into which the polynucleate sporogenous mass of protoplasm migrates to produce the resting spore. This is characterized by 9 to 15 branched haustoria in zonate arrangement between the equator and the distal pole.

A solution of the thinner cell walls in proximity to the young, advancing turbinate cells leads to the development of cavities in the hypertrophied tissue in which the resting spores are finally found inclosed.

The abundant development of the disease in the regions where it now occurs is apparently associated with excessive moisture during the period when infection is taking place. Any measures which can be taken to reduce the moisture near the surface of the soil at this time should reduce the disease.

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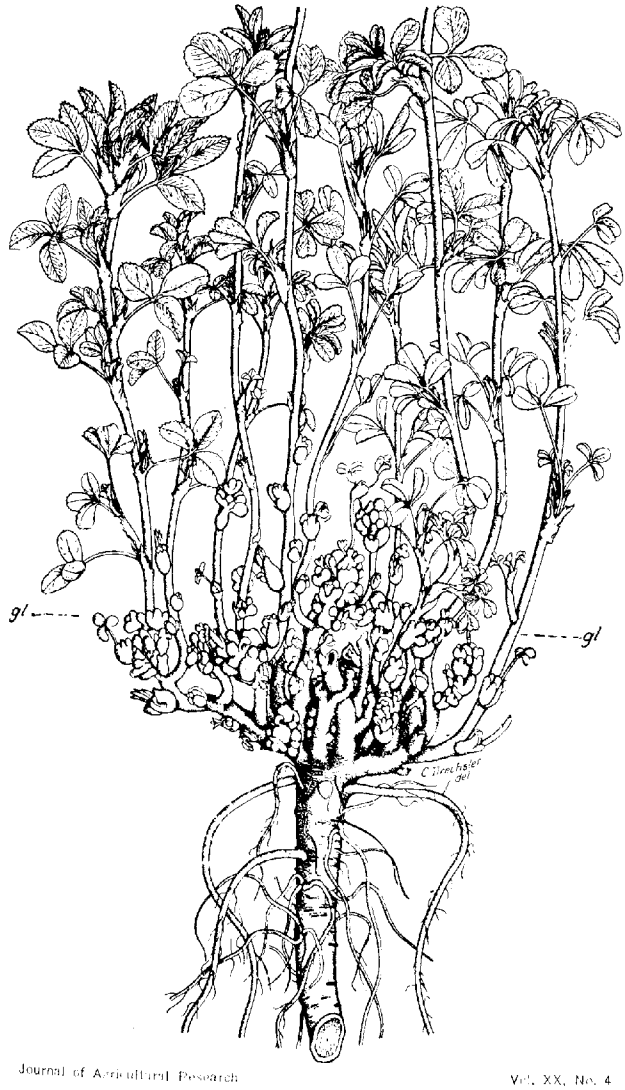
PLATE 47

Urophlyctis alfalfae:

Drawing of alfalfa plant, showing abundance of crownwart, as found early in May, 1919, in northern California. The dotted line *gl* represents the ground level. Varying degrees of abnormality in the development of the buds are shown. × ⅜.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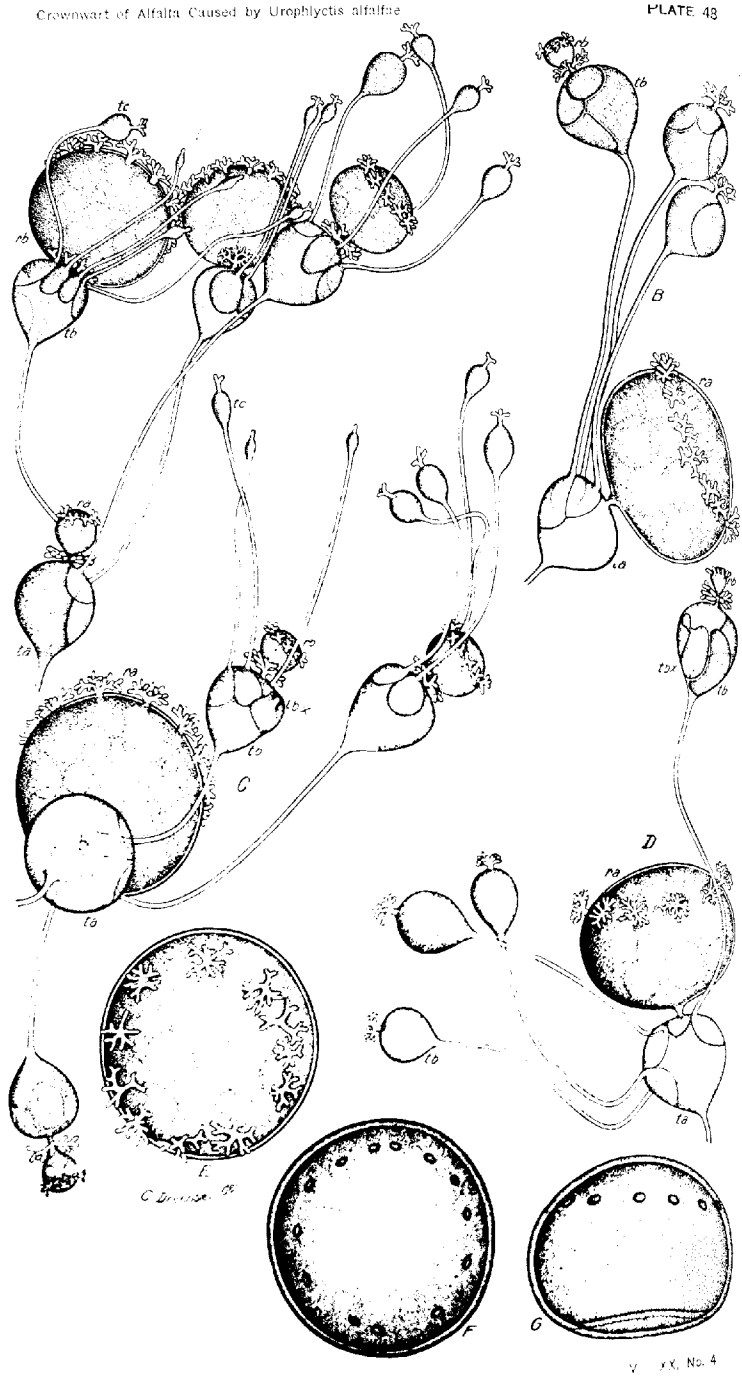


C. Dwight, del.

PLATE 48

Urophlyctis alfalfae.

A–D.—Peripheral portions of actively growing thallus of parasite dissected from living host: *ta, tb, tc,* turbinate cells of successive orders; *ra, rb,* resting spores produced by successive orders of turbinate cells; *tbx,* peripheral segments beginning to proliferate turbinate cells by budding. Note the single apical haustorium on the developing turbinate cells *tc;* its median position on the isthmus connecting turbinate cell *tb* and developing resting spore *rb;* its absence from isthmus between evacuated turbinate structure B, D, *ta,* and maturing resting spore B, D, *ra.* Note also zonate arrangement of haustoria between equator and distil pole of resting spore, A–D, *ra, rbs.*

E.—Nearly mature resting spore viewed from distil side, showing 11 haustoria in zonate arrangement.

F.—Mature resting spore viewed from distil pole, showing 13 pits that mark former location of haustoria.

G.—Mature resting spore viewed in profile, showing pits in zonate arrangement and light concavity on proximal side of spore.

Drawn with the aid of the camera lucida. Approxiately × 847.

PLATE 49

Urophlyctis alfalfae:

A.—Section of epidermal region of young foliar structures, showing young primary turbinate cells *ta-tg*, the first products of infection, within epidermal cells. Note attachment to cuticular wall by attenuated beak, increase in number of fungus nuclei during growth of turbinate cells, and pathological enlargement of host nuclei *hn*, in invaded cell, *hnx* being normal host nucleus.

B.—Section of young foliar element, showing wall of invaded epidermal cell disrupted and advance of secondary turbinate cells *tbc-tbe* into underlying tissue. One of the other secondary turbinate cells, *tbb*, is forcing its way down along the host cell wall, while another, *tba*, has been reflected toward the cuticular wall; *ta*, sporogenous cell of primary turbinate structure.

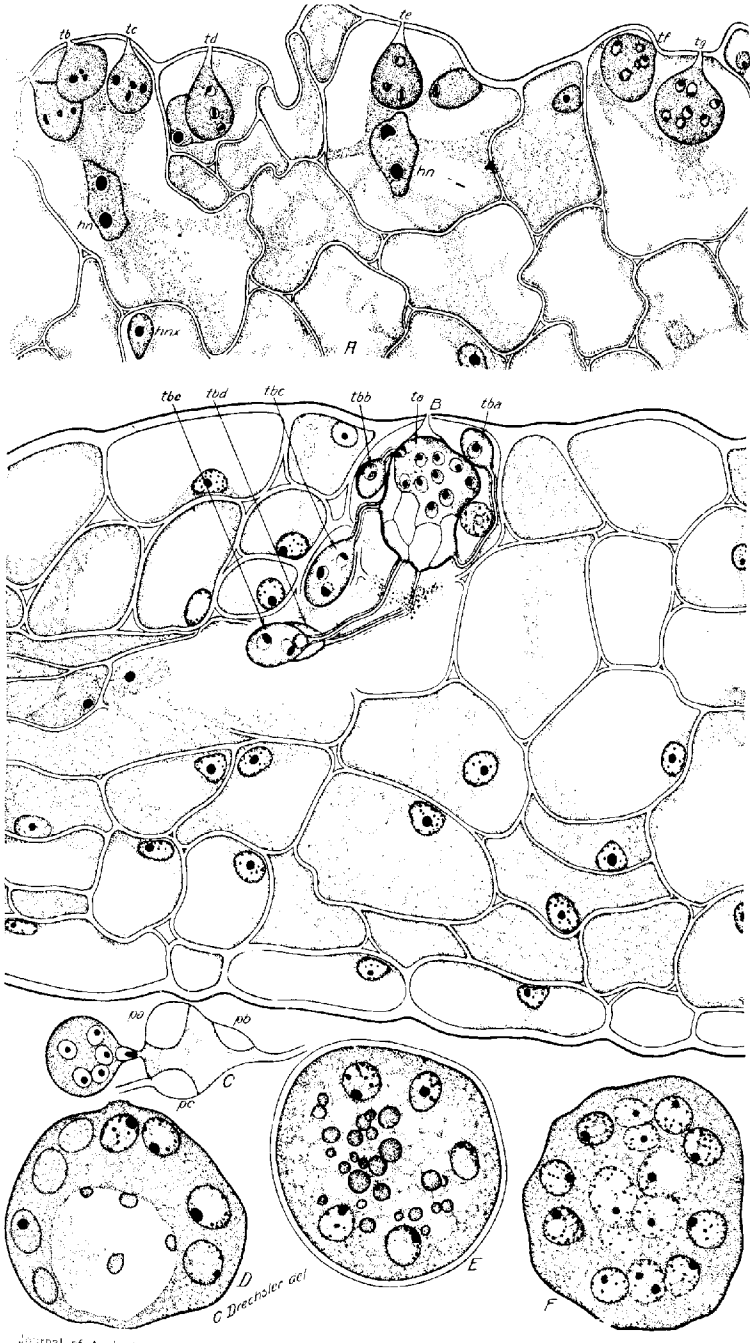
C.—Section of turbinate cell, showing 3 evacuated peripheral segments *pa-pc*. A nucleus is passing through the narrow isthmus connecting the nearly evacuated sporogenous cell with the resting spore, the elongated nucleole following the achromatin.

D.—Section of maturing resting spore, showing 8 nuclei and a central vacuole containing 4 granules staining red.

E.—Section of mature resting spore, showing numerous red-staining granules in center and 5 nuclei.

F.—Section of maturing resting spore, showing 11 normal nuclei and 4 enlarged nuclei in center, the latter apparently degenerating.

Drawn with the aid of the camera lucida. × 860.


tb
tc
td
te
tf
tg
hn
hnx
A
tbe
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tbc
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tba
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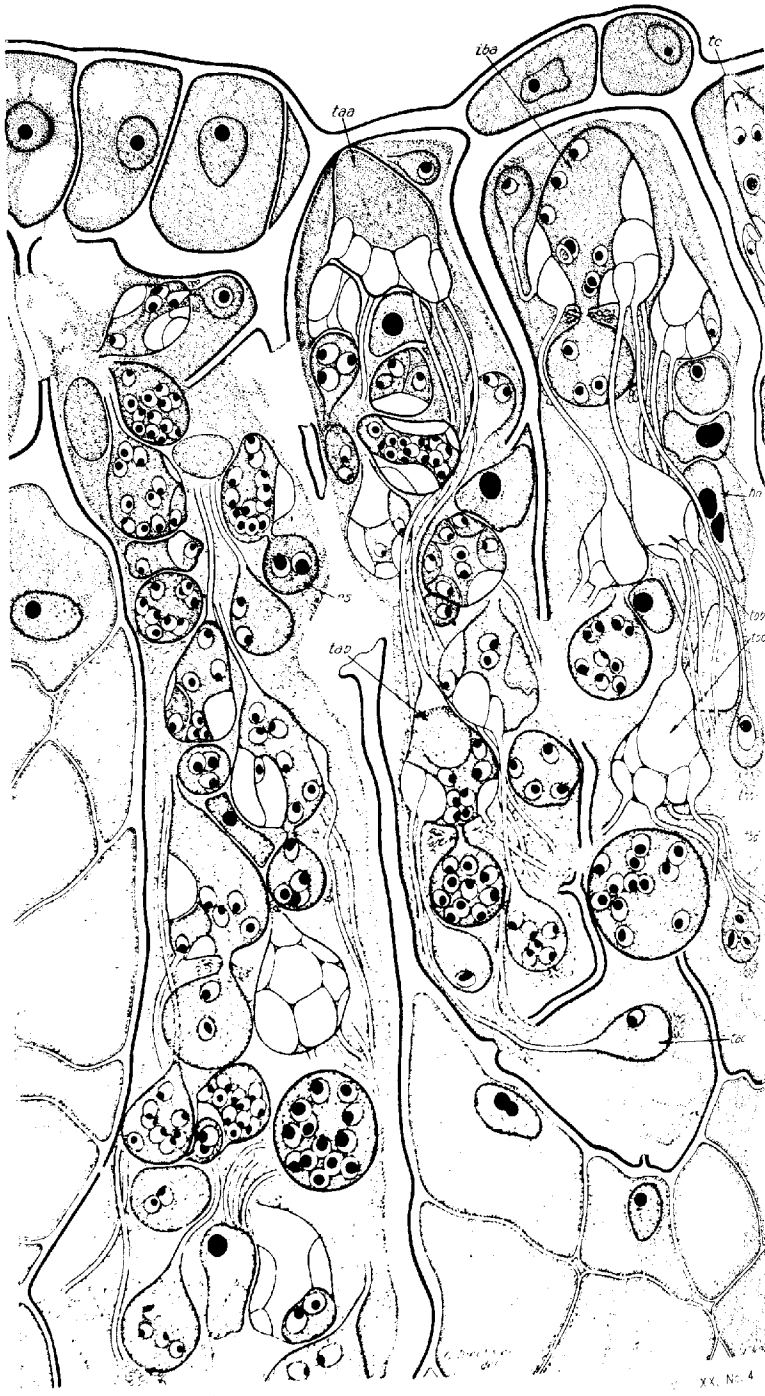

tba
tc
taa
ts
tap
tac

PLATE 50

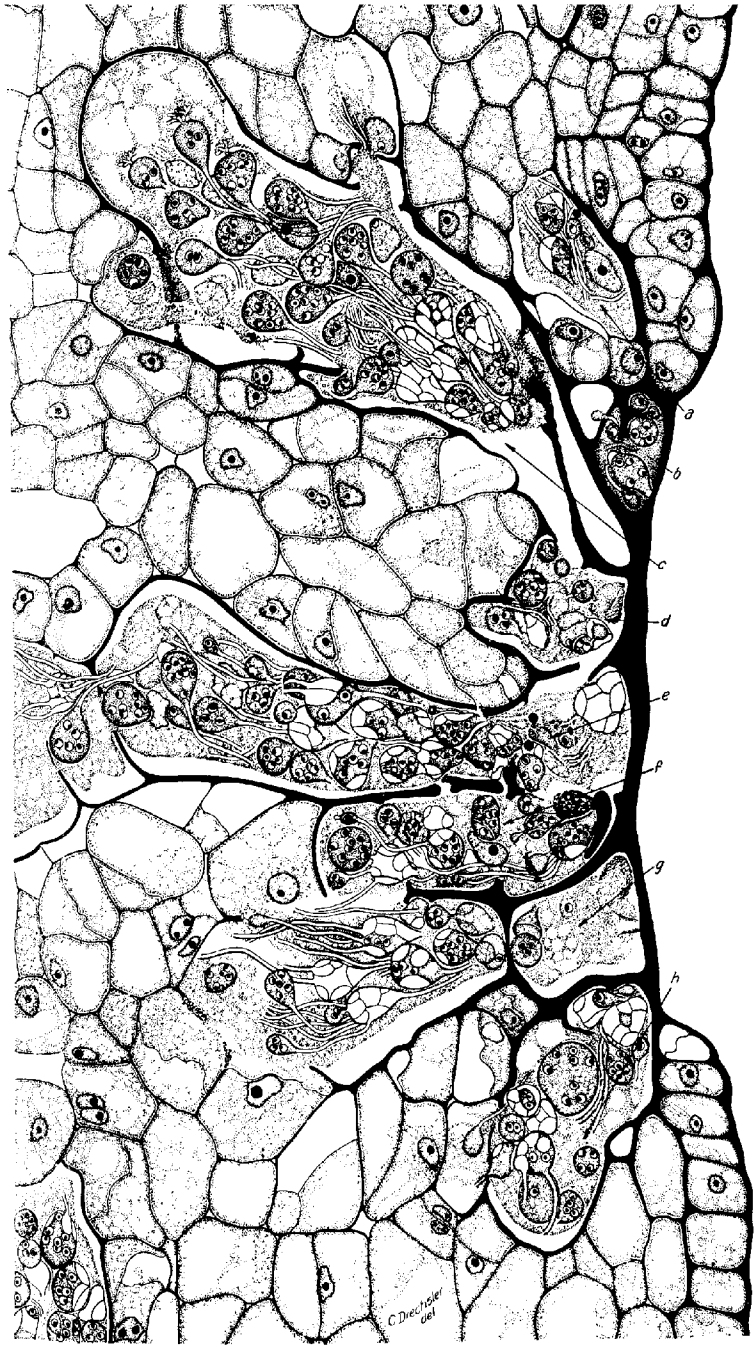
Urophlyctis alfalfae:

Section of diseased bud scale of alfalfa, showing four coalescing cavities, in three of which the large primary turbinate cells *taa*, *tba*, and *tc* may be distinguished; *taa* has not started to proliferate any resting spore, while the resting spore produced by *tba* is moderately young, although turbinate cells of later orders *tab*, *tbc*, and others have produced resting spores further along in development. The thickening of the host cell walls bounding the cavity and the enlargement of the host nuclei *hn* lying free within the cavity are conspicuous. Note also the large dimensions of the nucleus in the uninucleated turbinate cell *tbbx* and the relatively larger proportions of the nucleoles in the nuclei of the resting spore *rs*. Drawn with the aid of the camera lucida. × 860.

PLATE 51

Urophlyctis alfalfae:

Section of diseased bud scale attacked by *U. alfalfae*, showing a group of eight well-developed cavities *a-h* and their relation to the host tissue. Many of the cells adjacent to the cavities have divided at unusual angles, giving the tissue a characteristic appearance. In *b* the host cytoplasm and fungous material stain unusually deeply, as the result perhaps of general infiltration with some diffusing substance. Drawn with the aid of the camera lucida. Approximately × 417


a
b
c
d
e
f
g
h
C. Drechsler del.

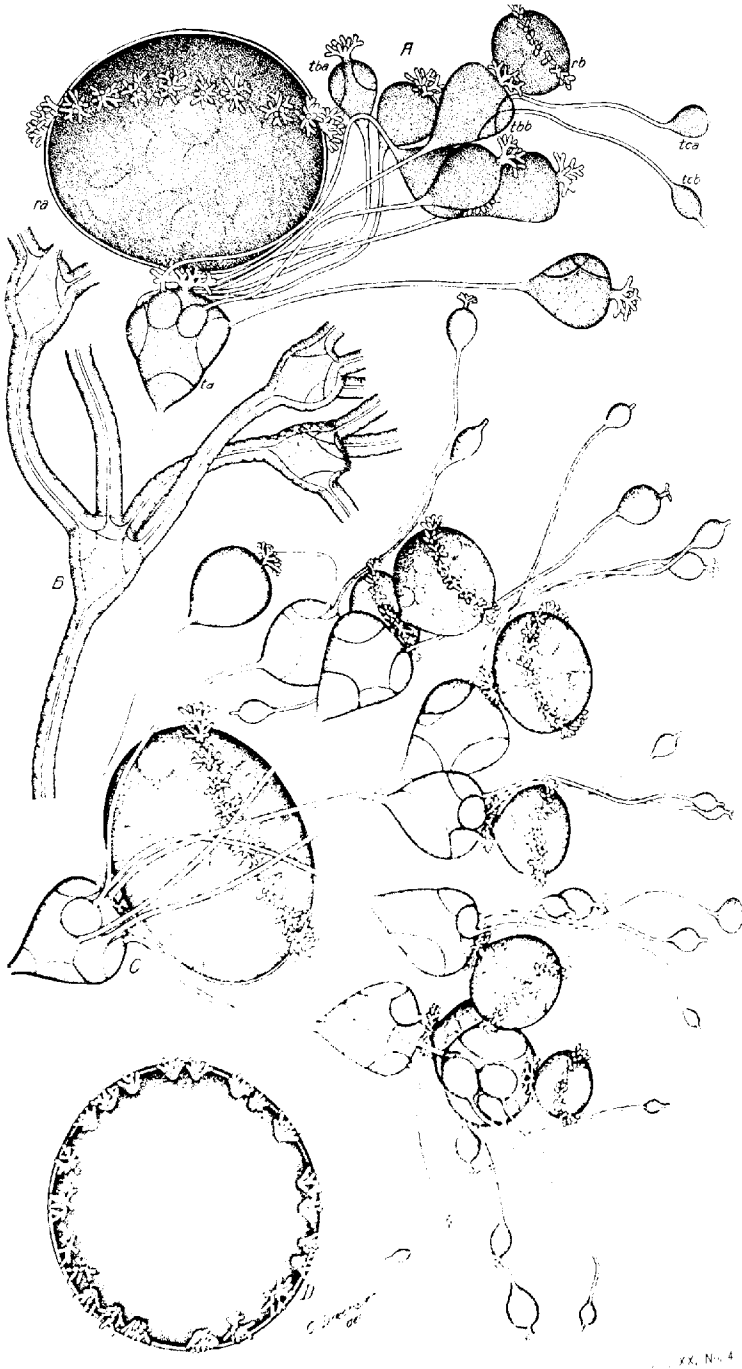

A
tba
rb
tbb
tca
tcb
ra
fa
Б
C

PLATE 52

A, C, D.—*Urophlyctis pluriannulatus*. B.—*Urophlyctis alfalfae*.

A.—Portion of actively growing thallus of *U. pluriannulatus* dissected from gall on leaf of *Sanicula menziesii*, including a turbinate cell *ta* with a nearly mature resting spore *ra; ta* is completely evacuated, having produced 7 turbinate cells of the next order, in one of which *tba* peripheral segments have been delimited, another, *tbb*, has produced two turbinate cells of the tertiary order *tca* and *tcb*, as well as a developing resting spore *rb*. Approximately × 847.

B.—Abnormally enlarged hyphae and turbinate cells of *U. alfalfae*, showing conspicuous thickening of the walls. × 860.

C.—Peripheral portion of actively growing thallus of *U. pluriannulatus*, similar to A, showing 8 turbinate cells of the second order, of which 7 have produced turbinate cells of the last order as well as resting spores. Approximately × 847.

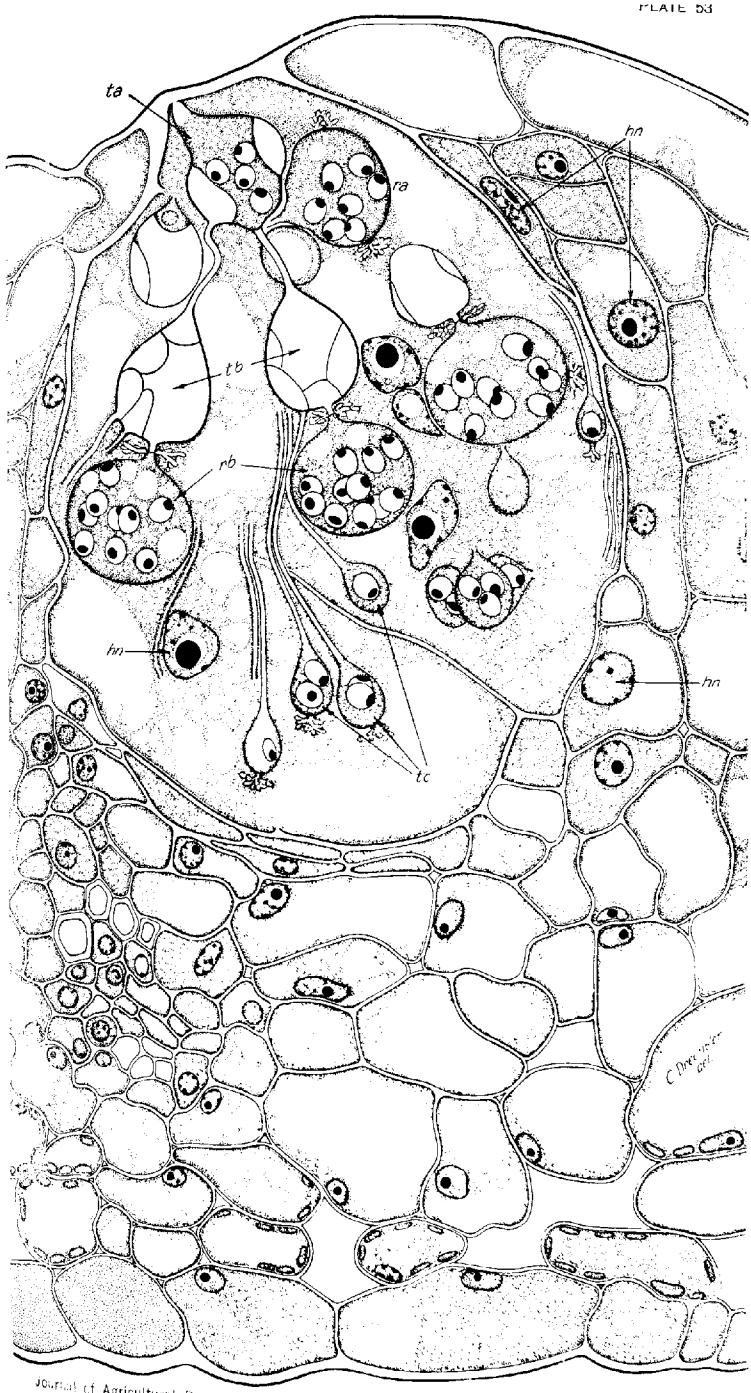
D.—Nearly mature resting spore of *U. pluriannulatus*, viewed from polar end, showing 22 haustoria in zonate arrangement. Approximately × 847.

Drawn with the aid of the camera lucida.

PLATE 53

Urophlyctis pluriannulatus:

Section of leaf of *Sanicula menziesii*, showing development of parasite within gall. Some of the fungus thallus appears to have dropped out of the section in the course of manipulations, as is indicated by the large unoccupied gaps; *ta*, primary turbinate cell; *tb*, *tc*, turbinate structures or cells of successive orders, the former completely evacuated, the latter in early first or second nucleated stage; *ra*, *rb*, resting spores produced by turbinate cells of successive orders; *hn*, host nuclei considerably enlarged as result of influence of parasite. Note the similarity in development of parasite to *U. alfalfae* and the relatively slight influence of parasitism on host anatomy. Drawn with the aid of the camera lucida. × 860.


ta
ra
hn
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Crownwart of Alfalfa Caused by Urophlyctis alfalfae PLATE 54

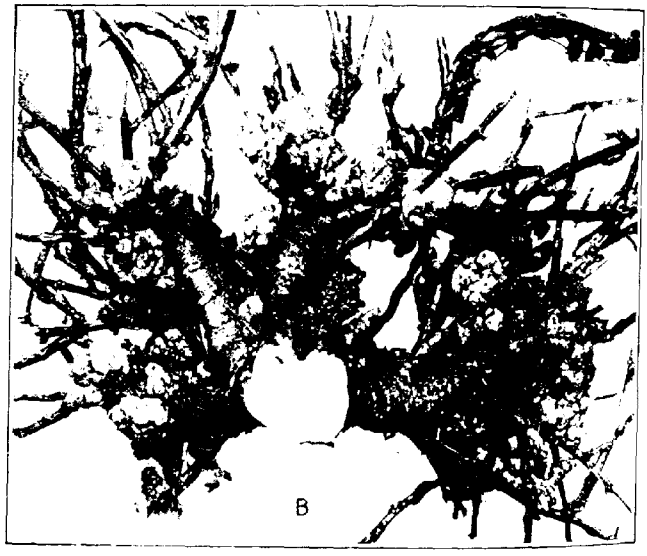


PLATE 54

Crowns of alfalfa plants bearing galls caused by *Urophlyctis alfalfae* photographed at different stages of development.

A.—A comparatively early stage of development at which the origin of the gall structures from the elements of developing buds can be traced.

B.—A later stage of development at which the origin of the tissue has become obscured. The tap root of this crown was cut off and the photograph taken from below. Galls usually become considerably larger than this before they begin to disintegrate if the plant continues vigorous growth.

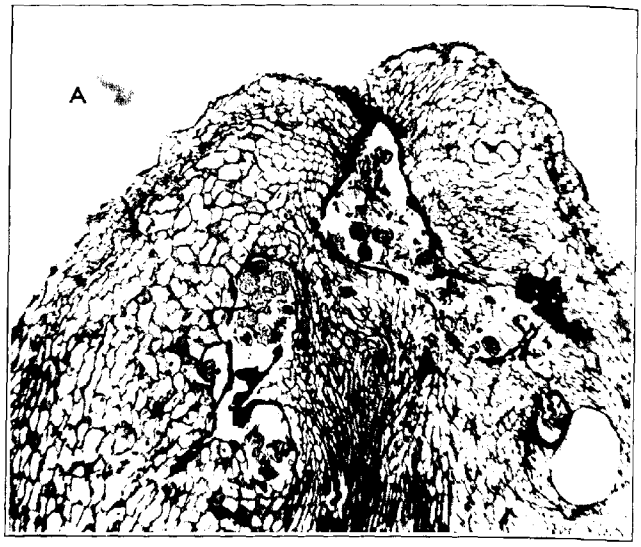
PLATE 55

A comparatively early stage of host reaction to invasion by *Urophlyctis alfalfae*. The cavities produced by the invading fungus can still be traced from the exterior into the parenchymatous tissue. A few of the cells which are about to be entered by the advancing fungus show some hypertrophy. The division of the cells beneath the epidermis has begun.

Crownwart of Alfalfa Caused by Urophlyctis alfalfae

PLATE 55

Crownwart of Alfalfa Caused by Urophlyctis alfalfae PLATE 56



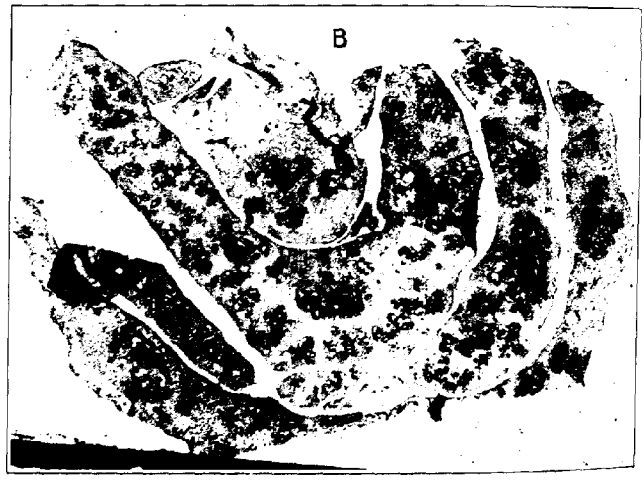


PLATE 56

A.—Late stage of development of host reaction to the invasion of *Urophlyctis alfalfae*. Infections have taken place near the tip of a growing point. The division of exterior cells has gone on extensively. The cells around the older portions of the cavity formed by the fungus have also been stimulated to division. The vascular bundle at the center of the mass of tissue has begun to produce parenchyma toward which the fungus is inclined to direct its course.

B.—Vertical section through a well-developed gall near its central axis, showing its laminated structure arising from the thickening of bud elements. The cavities containing the dark-colored spore masses are seen distributed through the tissue, a condition that causes the mottled appearance of the interior of the living gall.

PATHOLOGICAL ANATOMY OF POTATO BLACKLEG

By ERNST F. ARTSCHWAGER

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Blackleg, as has been shown by the researches of numerous investigators, notably Appel (*2*),[1] Smith (*7*), and Morse (*6*), is a bacterial disease affecting the underground part of the potato stem and the tubers. In its typical form the base of the potato stem shows a pronounced blackening which may extend several inches above the ground. The seed piece from which the diseased plants have been grown is found in a state of decay or is already completely destroyed by rot. The external symptoms are sufficiently striking to enable one to recognize diseased plants even at a distance. Such plants are of a lighter color and usually exhibit a xerophytic texture. They may be normal in size, but most often they are dwarfed and stocky, so that the disease is easily mistaken for leafroll. But while leafroll plants are firmly anchored in the ground, blackleg stems are easily pulled and always show the characteristic lesions on the underground part. The ease with which diseased plants are removed is so striking that one unconciously looks for a contributing mechanical cause, and that such a supposition is not altogether unfounded is shown in a note by Heygi (*4*) who reports wire-worm injuries in almost all the blackleg material that came under this observation. From the results of his investigations, Hegyi is inclined to consider the presence of the bacteria a secondary factor which has nothing to do with the original cause. Yet while it is true that wire worms may cause a loss of many potato hills, they are probably not responsible for the death of plants suffering from blackleg.

The xerophytic texture of the diseased plants is exhibited by stems and leaves alike. The foliage is discolored, usually light and of a metallic luster. The leaflets are folded, and the petiole and midrib are woody and lacking the elasticity and softness which characterize the normal organs. Not all the stems of a diseased plant are necessarily affected. Healthy sprouts may appear side by side with diseased ones (Pl. 57, A), and the diseased sprouts may exhibit various degrees of injury. Plants which have been attacked rather early often continue to live for a considerable period. These plants remain naturally dwarfed, the stalks are spindling, the internodes shortened, and the leaves small. Plants attacked at a more mature age may attain full size, though they usually succumb more quickly to the attack of the parasite than do many of the

[1] Reference is made by number (italic) to "Literature cited," p. 330.

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plants affected earlier in their life. In the last stages of the disease, when the rot has progressed far enough to cut off completely the water supply, the entire plant turns brown and sooner or later, depending on weather conditions, falls prey to the attack of saprophytic bacteria and fungi.

MATERIAL AND METHOD OF STUDY

The field from which the material for study was obtained is located in a clearing of the river bottom land near the Fort Lewis Mesa, Colo. The altitude is 7,500 feet. The soil is a sandy loam containing some organic matter and a water table sufficiently high to insure the growing of a crop without the customary irrigation. The tubers used were of Green Mountain and Rural New Yorker types. They were cut before planting, and because of the apparent soundness of the tubers no surface sterilization was attempted. The season was a normal one. The months of May and June were characterized by excessive dryness. Throughout July and August frequent showers insured a rapid growth of the plants. During the first week of August a severe hailstorm injured the foliage and stems so badly as to make further observations impracticable.

The first diseased plants appeared early in July. Their number increased during the following two weeks and then showed a decline on account of the death of a number of early infected plants and the reduction in number of new infections. Tubers of the same lot which had been disinfected and grown on irrigated mesa soil remained free from disease. The observations made at Fort Lewis were extended on material obtained from other parts of the State, especially the San Louis Valley. In every case the symptoms were similar, the only real difference being in the number of diseased plants per acre.

The plants taken for study were examined while fresh. For the purpose of completing microchemical work and checking results, suitable material was killed in Flemming's weaker solution and embedded in paraffin in the usual way. The principal reagents used were Haidenhein's haematoxylin-safranin stain for histological structures, Devaux's stain for pectic degeneration, phloroglucin-hydrochloric acid for lignification, and Altmann's acid fuchsin stain for protein crystals.

While all previous investigations on the blackleg disease deal with the morphology of the causal organism and its pathogenicity, this study has for its object a consideration of the pathological changes concomitant to the presence of the organism.

PATHOLOGICAL ANATOMY

In general, the histological changes consist in an increase of strongly lignified vascular tissue and in a transformation of part or most of the parenchyma cells of pith and cortex into sclereids (Pl. 58, B). Cytological abnormalities lie mainly in the occurrence of protein crystals in the parenchyma cells of the leaves, the stems, and the tubers.

The elements of the xylem are normal in size, though occasionally they appear smaller. The lumen is reduced; the walls are thicker and more strongly lignified. Even in unstained sections and without the microscope the xylem appears to be darkened. The discoloration often extends to the stem apex and into the petiole, but it is most pronounced in the underground parts of the stem where the external symptoms are most striking. Usually the cell wall alone is discolored, but sometimes a brown, gummy deposit is found in the lumen of the cells, especially of the larger vessels. In typical cases, only the primary xylem is affected; in advanced stages, however, a part of the secondary xylem may also show the browning of the walls. This discoloration of the elements of the xylem is not necessarily a symptom limited to blackleg, since it is associated with numerous other pathological disturbances and is commonly observed in plants which are suffering from excess of water.

The phloem fibers are more abundant. They, too, show a general increase in wall thickening and intensity of lignification. The secondary wall often is so thick as completely to fill the lumen (Pl. 57, C; 58, A); it is distinctly layered and contains numerous simple pits.

While one occasionally finds sclereids in the cortex of the underground stem of the normal plant, there is nothing that could compare with their relative abundance in plants suffering from blackleg. These sclereids are typical parenchyma cells with strongly lignified secondary walls (Pl. 57, C; 58, A). They are either scattered or form solid masses of tissue, often completely replacing the pith and part of the cortex. The transformation of pith cells into sclereids is most pronounced in the apical stem region and in the petiole. In the midrib and in the stem region close to the base, where the browning of the xylem is most pronounced, relatively few sclereids are found.

In the small parenchyma cells of the perimedullary zone similar changes occur. The cells show at first pectic degeneration, which is followed by lignification. The peripheral pith cells, especially in the interfascicular region, are sometimes completely transformed so that they form a sclerenchymatous sheath on the inner face of the vascular tissue.

The phloem elements are mostly normal at the base of the stem but show increasingly advanced pathological changes toward the apex and in the petiole. The cell walls are swollen, occasionally necrotic. The cells of the pericycle undergo similar changes which are more severe and are noticeable even in the lower stem regions.

Plants which are infected early but do not succumb to the attack of the parasite very readily show the most typical and pronounced symptoms. In plants in which the course of the disease has been a rapid one, relatively few changes are exhibited. It will be understood, however, that individual plants vary and that the environment, the age of the plant, and its physiological constitution will in a large measure determine the degree of anatomical changes in tissues and organs.

The presence and activity of the blackleg organism results in a gradual or rapid cutting off of the water supply from the roots and in a break in the path of translocation for plastic materials in the lower stem region. As a consequence of the decreased water supply, we have a decrease in growth activities, especially a check in elongation. The newly formed cells seem to mature more rapidly; in fact, mature and already strongly lignified cells are found close to the growing apex. As long as the leaves remain green and a minimum of water is insured synthesis of foods will go on, though less extensively than in healthy plants. There is not, however, an accumulation of starch as is commonly found in plants suffering from leafroll, but there is a utilization of the food in the laying down of extensive secondary thickenings in the cells of the xylem and fibers and in a transformation of parenchyma cells into thick-walled sclereids. Morse (6) reports that when the progress of the disease is slow—

> numerous aerial tubers will be formed on the stalks at the surface of the ground or in the axils of the leaves above.

It would be of interest to know whether in such a case the same anatomical changes occur which normally accompany blackleg.

Just as the formation of sclereids is the most pronounced histological symptom, the appearance of protein crystals in all organs of the plant, the leaves in particular, is a cytological phenomenon always associated with the disease. Protein crystals have been found in the tubers of normal plants. Bailey (1) reports the occurrence of cubical crystals in the tubers of *Solanum tuberosum*. A few years later Cohn (3) by the use of protein reactions identified the crystals of Bailey as belonging to the typical group. Heinricher (5) observed that in potato plants where the root system had been destroyed by decay the basal portions of the plant contained cubical protein crystals which were especially abundant in the cells of the phloem but were altogether absent from the cells of the epidermis and the collenchyma. Crystals have not been found in the aerial parts of the normal plant, and in the researches of the writer on the anatomy of the potato plant and the pathological anatomy of the leafroll disease they have not been observed. However, crystals have been noted by Stock (8) in aerial, axillary tubers, where they show the same distribution in peripheral cells of the cortex as do normally developed underground tubers. Protein crystals occur in great abundance in all organs of "blackleg" plants, especially in the leaves (Pl. 57, B; fig. 1). The crystals are usually cubical and vary in size from minute bodies to large structures with a diameter of two-thirds the size of a palisade cell. They are normally found in the cell sap or in the cytoplasm, very rarely inside the nucleus, although nuclear crystals, according to the extensive researches of Zimmermann (9), are not at all uncommon.

Nothing definite may be said in regard to the physiological importance of these structures. Crystals have been observed in many plants, in the fungi as well as the highly specialized angiosperms; but, while certain groups of plants show them in great abundance, other plant groups show just as conspicuous a lack. Heinricher (5) believed that the interception of the movement of plastic material to the roots causes a forcible deposition of the protein in the basal parts of the stem. This, however, could in itself not account for their formation as has already been pointed out by Stock (8), who observed protein crystals in aerial tubers but failed to find them in the stem, although the cells in the latter are completely filled with starch. The crystals probably constitute transitory food which may be used again in the metabolism of the plant and may accumulate when growth is inhibited unless an excess of photosynthetic products (as starch in the case of leafroll plants) stops protein synthesis altogether.

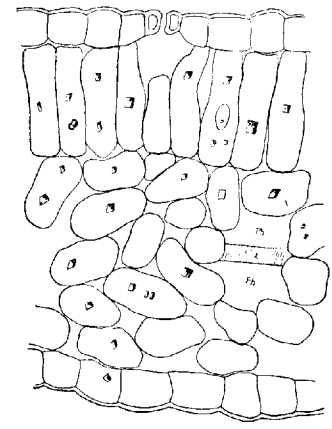

FIG. 1.—Section of potato leaf, showing distribution of protein crystals.

SUMMARY

(1) Potato plants affected with blackleg show an increase in strongly lignified vascular tissue and a transformation of part or most of the parenchyma cells of cortex and pith into sclereids.

(2) Associated with blackleg is the occurrence of protein crystals, especially in the cells of the leaves. Under normal conditions protein crystals have been observed only in the peripheral cell layers of the cortex of the potato tubers.

(3) Only diseased plants grown in the arid western parts of Colorado have been studied. It is possible that plants grown in the eastern United States and at a lower altitude do not exhibit the anatomical changes reported in this paper.

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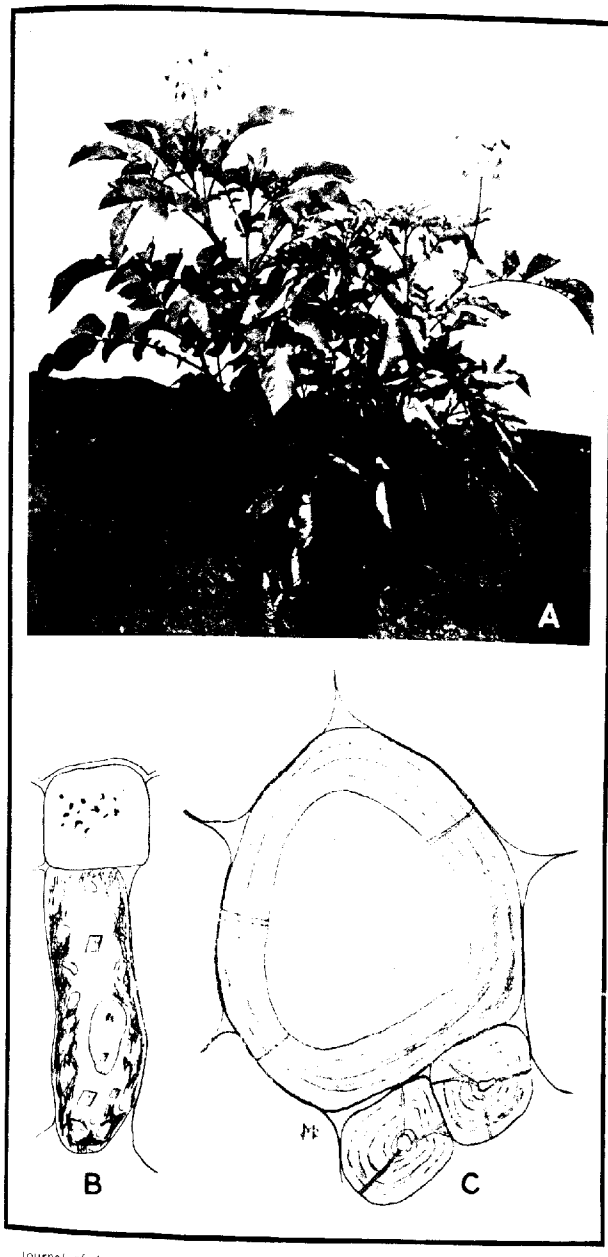
PLATE 57

A.—Plant affected with blackleg. One stem is healthy, while the other is severely diseased.

B.—Section of single upper epidermal cell of leaf and adjacent palisade cell. The epidermal cell is filled with granular tanniferous material; the palisade cell shows disorganized protoplasm, starch grains, and crystals. A small crystal is seen inside the nucleus.

C.—Section of pith cell which is transformed into a sclereid adjacent to phloem fibers. The walls of the latter are very thick and strongly lignified.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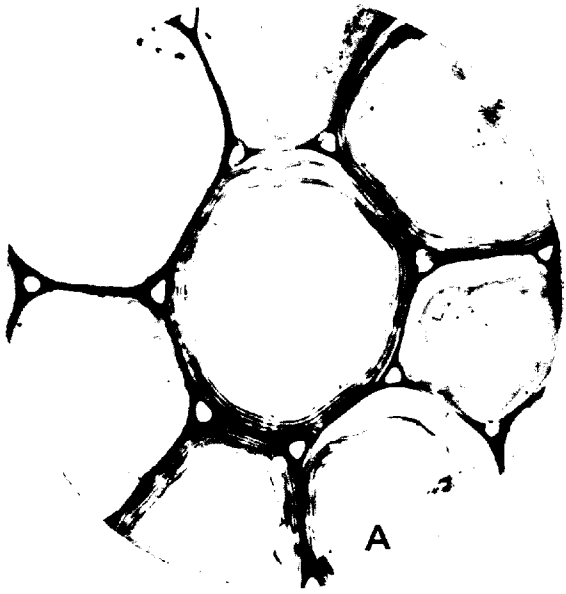
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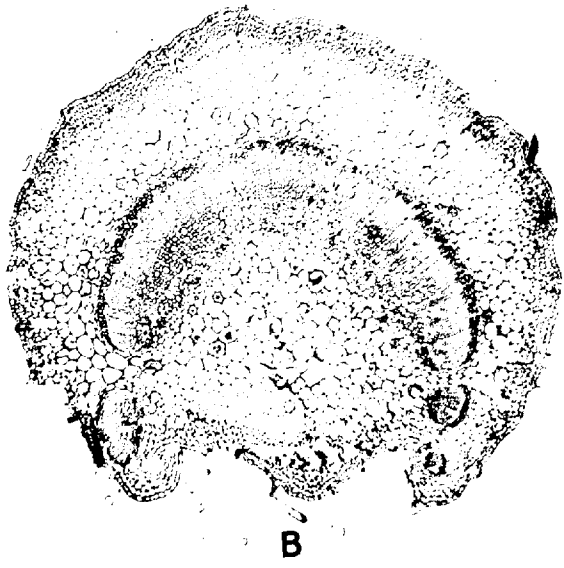
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PLATE 58

A.—Pith cells of petiole transformed into sclereids with typically stratified walls.

B.—Vascular tissue of the petiole greatly increased by blackleg. A number of sclereids are seen in the pith.

SCLEROTINIA MINOR, N. SP., THE CAUSE OF A DECAY OF LETTUCE, CELERY, AND OTHER CROPS

By IVAN C. JAGGER

Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

Smith [1] (1900) recorded the occurrence of a fungus similar to *Sclerotinia libertiana* Fuckel, which, however, produced much smaller sclerotia (Pl. 59, A) in greenhouses of Massachusetts, where it was causing a destructive rot of lettuce. Duggar [2] (1909) states that a similar fungus occurs on lettuce in the vicinity of both Boston and New York City. In 1911 the writer [3] obtained what appeared to be the same fungus from decayed lettuce grown in the vicinity of New York. It was collected in 1912 and again in 1914 at South Lima in western New York, where it seemed to be well established and was causing considerable injury to lettuce grown on muck soil. In 1914 it was also collected on lettuce in a greenhouse at Rochester, N. Y., but the fungus was not again found in that vicinity, although numerous collections of diseased lettuce were made during the next three years. In the fall of 1919 Dr. W. S. Beach of the Pennsylvania Agricultural Experiment Station advised that the fungus is frequently found on celery and lettuce in the vicinity of Philadelphia. During the winter season of 1919–20 the writer observed the fungus in destructive amounts in a single field of lettuce at Sanford, Fla. In numerous inspections of lettuce in that vicinity throughout the season the fungus was observed in no other fields, although *S. libertiana* was more or less abundant in all fields. This suggests that the fungus forming small sclerotia may have been recently introduced into that section.

The fungus causes a very rapid decay and collapse of growing lettuce plants. The disease produced is almost identical with that caused by *S. libertiana*. A soft, watery decay may begin at any point on the plant but usually on the lower leaves, which rest on the ground, or on the stem near the ground. The rot spreads very rapidly, and usually the main stem and bases of the leaves are soon involved. The result is a rather sudden collapse of the whole plant. The plant is rapidly converted to a soft, watery mass. When the decayed mass is pulled apart the spaces between and around the decayed leaves and stem are found to

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be filled with white wefts of mycelium, which in a few days are replaced by numerous small black sclerotia. General observations indicate that the fungus possibly causes a rather more rapid decay and collapse of plants than is caused by *S. libertiana*. The wefts of white mycelium in decaying plants are less conspicuous, and the sclerotia are much smaller and much more numerous than in plants attacked by *S. libertiana*.

On several occasions bits of culture media covered with mycelium of the fungus have been placed on growing lettuce plants. When moist conditions have followed the inoculation, characteristic rapid decay has invariably resulted. Prof. H. H. Whetzel has found that the fungus is capable of attacking a large number of plants, data on which will be

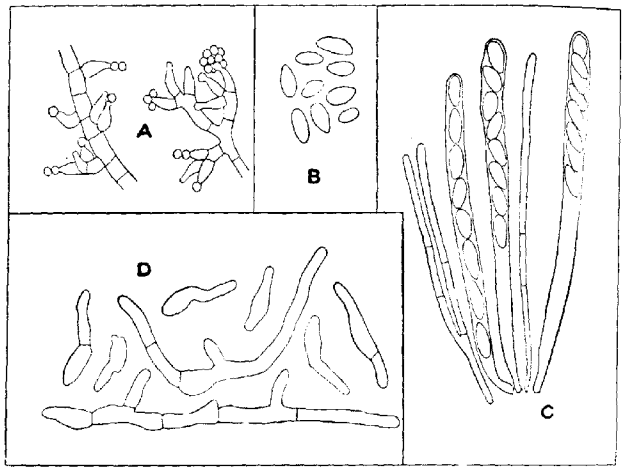


FIG. 1.—Camera lucida drawings of *S. minor*: A, microconidia and conodiophores; B, ascospores; C, germinating ascospores; D, asci and paraphyses.

published in connection with his studies of the genera Sclerotinia and Botrytis.

Strains of the fungus isolated from lettuce grown in the vicinity of New York, Rochester, and South Lima, N. Y., Philadelphia, Pa., and Sanford, Fla., have been grown in parallel cultures on various media and have in every case appeared to be identical. Apothecia produced by the three strains from New York State have shown neither macroscopic nor microscopic differences.

Apothecia (Pl. 59, B, C) have several times developed from sclerotia which had been allowed to age on unsterilized sand for from 4 to 12 months and which were then held under moist and well-lighted conditions. Studies of fresh mature apothecia were made in 1912, 1914, and 1917 (fig. 1). Measurements of spores, asci, and paraphyses in the

description are from the combined data of the three years, since the three sets of data agree very closely. Microconidia (fig. 1) have appeared in abundance on a medium consisting of a 2 per cent agar flour in distilled water. Cultures have been obtained repeatedly from single ascospores which have shown the apothecia to be the fruiting stage of the sclerotia-producing fungus.

Smith (1900) [1] in studies of this fungus was unable to obtain apothecia, although apothecia of *S. libertiana* were obtained in abundance. In hundreds of cultures the fungus developed only the characteristic small sclerotia, but in a single culture the small sclerotia at first appeared, and later the characteristic large sclerotia of *S. libertiana* appeared among the small ones. Smith believed that *S. libertiana* developed directly from the small sclerotia and, therefore, concluded that the fungus is—

> a degenerate form of *S. libertiana* which has almost entirely lost the ability to reproduce by spores.

The repeated development during several years of characteristic apothecia and the fact that during 10 years numerous cultures of several strains of the fungus have shown very uniform characteristics seem sufficient grounds for considering the fungus a distinct species. As it seems to agree with no described species, the following description is given.

Sclerotinia minor, n. sp.

Apothecia one, rarely more, from a single sclerotium; disc saucer-shaped, 0.5 to 2 mm. in diameter; stalk cylindrical, slender, flexuous, attenuated downward, 5 to 12 mm. long; asci cylindrical to cylindro-clavate, 125 to 175 μ by 8 to 11 μ, average of 30 measurements 141 by 8.9 μ; spores 8, ellipsoid to ovoid, hyaline, 5 to 8.8 μ by 8.3 to 19.9 μ, average size of 200 spores 7 by 14.1 μ with over 80 per cent 6 to 8 μ by 12 to 16 μ; paraphyses filiform to cylindro-clavate, septate, rarely branched, same length as asci, 3 to 4 μ in diameter; microconidia globose, hyaline, 3 to 4.2 μ, borne apically on short obclavate conidiophores; appressoria abundant; sclerotia black, irregular, 0.5 to 2 mm. in diameter, often anastomosing to form irregular flattened bodies several millimeters in length.

Parasitic on lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), and other plants: distribution, Massachusetts, New York, Pennsylvania, and Florida.

SUMMARY

Sclerotinia minor, n. sp., produces a decay of lettuce and other plants similar to that produced by *S. libertiana*. It is known to occur in Massachusetts, New York, Pennsylvania, and Florida.

[1] SMITH, Ralph E. OP CIT.

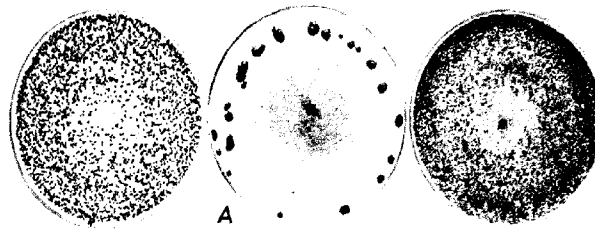
PLATE 59

A.—Sclerotia on hard potato agar: center, *Sclerotinia libertiana*, either end, *S. minor*.

B.—Apothecia of *S. libertiana*.

C.—Apothecia of *S. minor*.

Note relative size of apothecia in B and C.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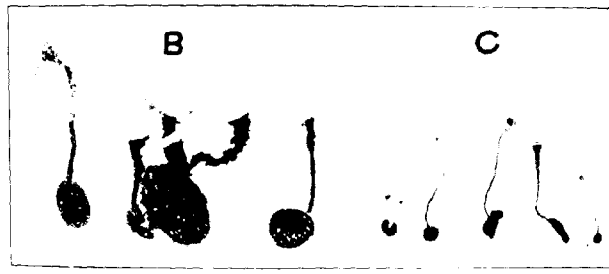
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